Immunohistochemistry in Undifferentiated Neoplasm/Tumor of Uncertain Origin

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• ***Context.***—**Immunohistochemistry has become an indispensable ancillary study in the identification and classification of undifferentiated neoplasms/tumors of uncertain origin. The diagnostic accuracy has significantly improved because of the continuous discoveries of tissue-specific biomarkers and the development of effective immunohistochemical panels.**

Objectives.—**To identify and classify undifferentiated neoplasms/tumors of uncertain origin by immunohistochemistry.**

Data Sources.—**Literature review and authors' research data and personal practice experience were used.**

Conclusions.—**To better guide therapeutic decisions and predict prognostic outcomes, it is crucial to differentiate the specific lineage of an undifferentiated neoplasm. Application of appropriate immunohistochemical panels enables the accurate classification of most undifferentiated neoplasms. Knowing the utilities and pitfalls of each tissue-specific biomarker is essential for avoiding potential diagnostic errors because an absolutely tissue-specific biomarker is exceptionally rare. We review frequently used tissue-specific biomarkers, provide effective panels, and recommend diagnostic algorithms as a standard approach to undifferentiated neoplasms.**

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After an extensive workup, combining clinical, radiologic, morphologic, and immunohistochemical (IHC) findings, we suggest, as have others,[1] that a truly undifferentiated neoplasm is rare. In this review article, we use the term *undifferentiated neoplasm/tumor of uncertain origin* to describe a tumor that lacks a specific-lineage differentiation or a tumor with a specific-lineage differentiation but an uncertain primary origin if based on morphologic features alone. In daily practice, most undifferentiated neoplasms/tumors of uncertain origin are eventually determined to be carcinomas/sarcomatoid carcinomas. Therefore, the methods for determining the origin of a carcinoma/sarcomatoid carcinoma will be the primary focus of this review article.

The differential diagnosis of the origin of a carcinoma usually includes the lung, breast, kidney, ovary, uterus, upper gastrointestinal (GI) tract, pancreatobiliary tract, urinary tract, thyroid, prostate, liver, and adrenal gland. Numerous biomarkers have been studied and suggested as useful for that purpose,[1–68] such as cytokeratin (CK) 7, CK20, estrogen receptor (ER), gross cystic disease fluid protein 15 (GCDFP-15), mammaglobin (MGB), thyroid transcription factor 1 (TTF1), napsin A, caudal type homeobox 2 (CDX2), Wilms tumor 1 (WT1), OCT4 (octamer-binding transcription factor 4), and paired box gene (PAX) 8. More recently, GATA binding protein 3 (GATA3), arginase 1 (ARG1), trefoil factor (TFF) 1, ankyrin repeat domain 30A (NY-BR-1), sal-like protein 4 (SALL4), special AT-rich sequence-binding protein 2 (SATB2), cadherin-17 (CDH17), and von Hippel-Lindau tumor suppressor (pVHL) have been reported as useful as well.[69–108] However, no single antibody is absolutely sensitive and specific for a specific entity. Obviously, the selection of an appropriate IHC panel is crucial to reaching a correct diagnosis, and the question is how to determine which is the most effective panel for a specific differential diagnosis. How can one avoid the overuse and underuse of available markers?

In this review article, we (1) propose working algorithms as a standard approach to identifying and classifying undifferentiated neoplasms/tumors of uncertain origin with a focus on carcinomas; (2) review the utilities and pitfalls of frequently used and recently described biomarkers, such as TTF1, napsin A, ER, GATA3, OCT4, SALL4, ARG1, TFF1, CDX2, SATB2, CDH17, and pVHL; (3) refine the most effective IHC panels for the differential diagnosis of carcinomas with different cytokeratin expression profiles ($CK7^+/CK20^-$, $CK7^+/CK20^+$, $CK20^+/CK7^-$, and $CK7^-/CK20^-$); and (4) suggest a diagnostic panel of antibodies for different entities, such as small blue cell tumors, spindle cell tumors, epithelioid tumors, and pleomorphic tumors, if based on morphologic features alone.

HOW TO APPROACH UNDIFFERENTIATED NEOPLASMS/TUMORS OF UNCERTAIN ORIGIN

A Step-by-Step Approach

There are many ways to work up an undifferentiated neoplasm. The following is a brief summary of our strategies to approach an undifferentiated neoplasm/tumor of uncertain origin (see Figure 1).

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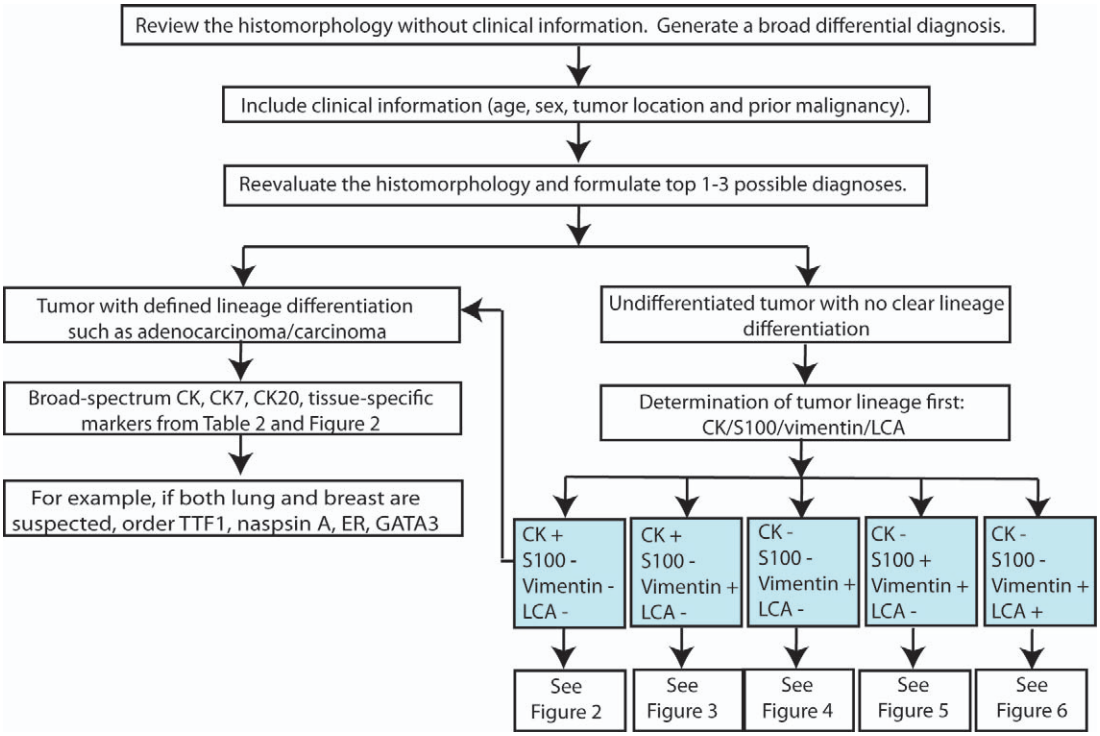

Figure 1. *The diagnostic algorithm for workup of undifferentiated neoplasms. Abbreviations: CK, cytokeratin; ER, estrogen receptor; GATA3, GATA binding protein 3; LCA, leukocyte common antigen; TTF1, thyroid transcription factor 1.*

1. **Review the Slides Without Knowing any Clinical Information.**—Morphologic features are fundamental. The first step is to determine whether the lesion is malignant. If a benign/reactive condition is included in the differential diagnosis, caution should be taken when applying any immunostains because IHC may or may not contribute to that process or may lead one to the wrong conclusion. If the lesion is malignant, it is important to review the slides and generate a broad differential diagnosis based on the morphologic features alone. One can be misled by incomplete or inaccurate clinical information.
2. **Consider the Basic Clinical Information, Such as Age, Sex, Tumor Location, and Prior Malignancy.**—After formulating the initial differential diagnostic categories, consider the patient's age, sex, tumor location, and any prior malignancy. Follow the statistics, and focus on the common entities in that particular age group of patients and tumor locations. Jumping to a conclusion of an uncommon entity in the initial diagnostic workup is not a wise choice. For instance, in a 60-year-old man with lytic bone lesions, the primary differential diagnosis should include a metastatic carcinoma from the lung, prostate, kidney, thyroid, or upper GI tract. A primary osteosarcoma or other sarcomas in that age group is rare and should not be included in the initial diagnostic consideration. In contrast, if a 10-year-old boy presents with a lytic bone lesion, osteosarcoma, Ewing sarcoma/primitive neuroectodermal tumor, eosinophilic granuloma, and benign/reactive conditions, such as osteomyelitis should be considered first. A metastatic carcinoma would be unlikely in that age group.
3. **Reevaluate Morphologic Features of the Tumor and Predict the Most Likely Category, Such as Carcinoma, Melanoma, Sarcoma, Lymphoma, or Germ Cell Tumor.**—Based on the patient's age, sex, tumor location, prior malignancy, and morphologic features, narrow down the initial differential diagnosis to 1 to 3 options, if possible. For example, is this a carcinoma? Is this an adenocarcinoma (ADC)? If it is an ADC, what is the likely primary site? Based on the tumor morphology, patient's age, and tumor location, Sheahan et al[109] demonstrated that pathologists were able to correctly identify the tumor origin as their first choice in 50% to 55% of cases or as their first, second, or third choice in 67% to 74% of cases.[110]
4. **Determine the First Diagnostic IHC Panel to Order.**—There are 2 likely scenarios. In the first scenario, there is clear lineage differentiation, such as an ADC/carcinoma, and the next step will be determining the likely primary site. A broad-spectrum cytokeratin cocktail (AE1/3 and CAM 5.2), CK7, CK20, plus relatively organ-specific markers from Table 1 and Figure 2, a through d, should be included in the initial diagnostic panel. For example, if a lung primary is suspected, TTF1 and napsin A should be included. If both lung and breast primaries are suspected, then TTF1, napsin A, ER, and GATA3 should be considered. The second scenario is an undifferentiated neoplasm with no clear lineage differentiation, but one in which a carcinoma is suspected; however, other categories, such as melanoma, sarcoma, and lymphoma cannot be entirely ruled out. In this case, the process is to determine the likely category of that tumor. The initial IHC panel would include markers to cover a broad category of neoplasms, such as carcinoma, melanoma, sarcoma, and lymphoma. The algorithms for workup of a tumor of uncertain origin/undifferentiated neoplasm are summarized in Figures 1 through 6.

Table 1. Useful Markers for Identifying Tumor Origin	
Primary Site	**Markers**
Adrenal cortical neoplasm	Mart-1, inhibin-α, calretinin, SF-1
Alveolar soft part sarcoma	TFE3
Angiomyolipoma	HMB-45, SMA
Atypical lipomatous tumor	MDM2 (MDM2 by FISH is a more sensitive and specific test), CDK4
Breast carcinoma	GATA3, ER, GCDFP-15, TFF1, MGB
Chordoma	Cytokeratin, S100
Choriocarcinoma	β-HCG, CD10
Desmoplastic small round cell tumor	Cytokeratin, CD99, desmin, WT1 (N-terminus)
Embryonal carcinoma	SALL4, LIN28, OCT4, NANOG, CD30, SOX2
Endocervical adenocarcinoma	PAX8, p16, CEA, HPV ISH, loss of PAX2
Endometrial adenocarcinoma	PAX8/PAX2, ER, vimentin
Endometrial stromal sarcoma	CD10, ER
Epithelioid sarcoma	CD34, loss of INI1
Ewing sarcoma/PNET	CD99, Fli-1, NKX2-2
Follicular dendritic cell tumor	CD21, CD35
Gastrointestinal stromal tumor	CD117, DOG1
GI tract, lower	CDH17, SATB2, CDX2, CK20
GI tract, upper	CDH17, CDX2, CK20
Hepatocellular carcinoma	ARG1, glypican-3, HepPar-1, AFP
Histiocytosis X	CD1a, S100
Hyalinizing trabecular adenoma of the thyroid	MIB-1 (unique membranous staining pattern)
Intrahepatic cholangiocarcinoma	pVHL, CAIX
Low-grade fibromyxoid sarcoma	MUC4
Lung adenocarcinoma	TTF1, napsin A
Mast cell tumor	CD117, tryptase
Melanoma	S100, mart-1, HMB-45, MiTF, SOX10, PNL2
Merkel cell carcinoma	CK20 (perinuclear dot staining), MCPyV
Mesothelial origin	Calretinin, WT1, D2-40, CK5/6, mesothelin
Myeloid sarcoma	CD43, CD34, MPO
Myoepithelial carcinoma	Cytokeratin and myoepithelial markers. May lose INI1
Myxoid and round cell liposarcoma	NY-ESO-1
Neuroendocrine origin	Chromogranin, synaptophysin, CD56,
Ovarian clear cell carcinoma	pVHL, HNF-1β, KIM-1, PAX8
Ovarian serous carcinoma	PAX8, ER, WT1
Pancreas, acinar cell carcinoma	Glypican-3, antitrypsin
Pancreas, ductal adenocarcinoma	MUC5AC, CK17, maspin, S100P, IMP3
Pancreas, neuroendocrine tumor	PR, PAX8, PDX1, CDH17, islet-1
Pancreas, solid pseudopapillary tumor	Nuclear β-catenin, loss of E-cadherin, PR, CD10, vimentin
Papillary RCC	P504S, RCCma, pVHL, CD10, PAX8, KIM-1
Prostate, adenocarcinoma	PSA, PSAP, ERG, NKX3.1
RCC, clear cell type	PAX8/PAX2, RCCma, pVHL, CD10, KIM-1
Rhabdomyosarcoma	Myogenin, desmin, MyoD1
Salivary duct carcinoma	GATA3, AR, GCDFP-15, Her-2/neu
Seminoma	SALL4, LIN28, OCT4, CD117, D2-40
Sex cord stromal tumors	SF-1, inhibin-α, calretinin, FOXL2
Smooth muscle tumor	SMA, MSA, desmin, calponin
Solitary fibrous tumor	CD34, BCL2, CD99
Squamous cell carcinoma	p40, CK5/6, p63, SOX2, desmocollin-3
Synovial sarcoma	TLE1, cytokeratin
Thymic origin	PAX8, p63, CD5
Thyroid follicular cell origin	TTF1, PAX8, thyroglobulin
Thyroid medullary carcinoma	Calcitonin, TTF1, CEA
Translocational RCC	TFE3
Urothelial carcinoma	GATA3, UPII/UPIII, S100P, CK5/6, CK903, p63, CK20
Vascular tumor	ERG, CD31, CD34, Fli-1
Yolk sac tumor	SALL4, LIN28, glypican-3, AFP

Abbreviations: AFP, α-fetoprotein; AR, androgen receptor; ARG1, arginase-1; Bcl2, B-cell chronic lymphocytic leukemia/lymphoma 2; β-HCG, β-human chorionic gonadotropin; CAIX, carbonic anhydrase IX; CD, cluster of differentiation; CDH17, cadherin-17; CDK4, cyclin-dependent kinase 4; CDX2, caudal type homeobox 2; CEA, carcinoembryonic antigen; CK, cytokeratin; D2-40, podoplanin; DOG1, discovered on GIST-1; ER, estrogen receptor; ERG, ETS-related gene; FISH, fluorescence in situ hybridization; Fli-1, friend leukemia virus integration 1; FOXL2, forkhead box L2; GATA3, GATA binding protein 3; GCDFP-15, gross cystic disease fluid protein 15; GI, gastrointestinal; HepPar-1, hepatocyte paraffin-1; HMB-45, human melanoma black 45; HNF-1β, hepatocyte nuclear factor 1 beta; HPV, human papillomavirus; IMP3, IMP3, insulin-like growth factor II messenger RNA binding protein-3; INI1, integrase interactor 1; ISH, in situ hybridization; KIM-1, kidney injury molecule 1; LIN28, lin-28 homolog A; Mart-1, melanoma-associated antigen recognized by T-cells 1; maspin, mammary serine protease inhibitor; MCPyV, Merkel cell polyomavirus; MDM2, mouse double minute 2 homolog; MGB, mammaglobin; MIB-1, mindbomb homolog 1; MiTF, microphthalmia-associated transcription factor; MPO, myeloperoxidase; MSA, muscle-specific actin; MUC, mucin; MyoD1, myogenic differentiation 1; NANOG, NANOG homeobox; NKX2-2, NK2 homeobox 2; NKX3.1, NK3 homeobox 1; NY-ESO-1, cancer/testis antigen 1B; OCT4, octamer-binding transcription factor 4; p504S, α-methylacyl-CoA racemase; PAX, paired box gene; PDX1, pancreatic duodenal homeobox 1; PNET, primitive neuroectodermal tumor; PNL2, melanoma-associated antigen PNL2; PR, progesterone receptor; PSA, prostate-specific antigen; PSAP, prostate-specific acid phosphatase; pVHL, von Hippel-Lindau tumor suppressor; RCC, renal cell carcinoma; RCCma, renal cell carcinoma marker; S100P, placental S100; SALL4, sal-like protein 4; SATB2, special AT-rich sequence-binding protein 2; SF-1, steroidogenic factor 1; SMA, smooth muscle actin; SOX, sex-determining region Y box; TFE3, transcription factor E3; TFF1, trefoil factor 1; TLE1, transducin-like enhancer of split 1;TTF1, thyroid transcription factor 1; UP, uroplakin; WT1, Wilms tumor 1.

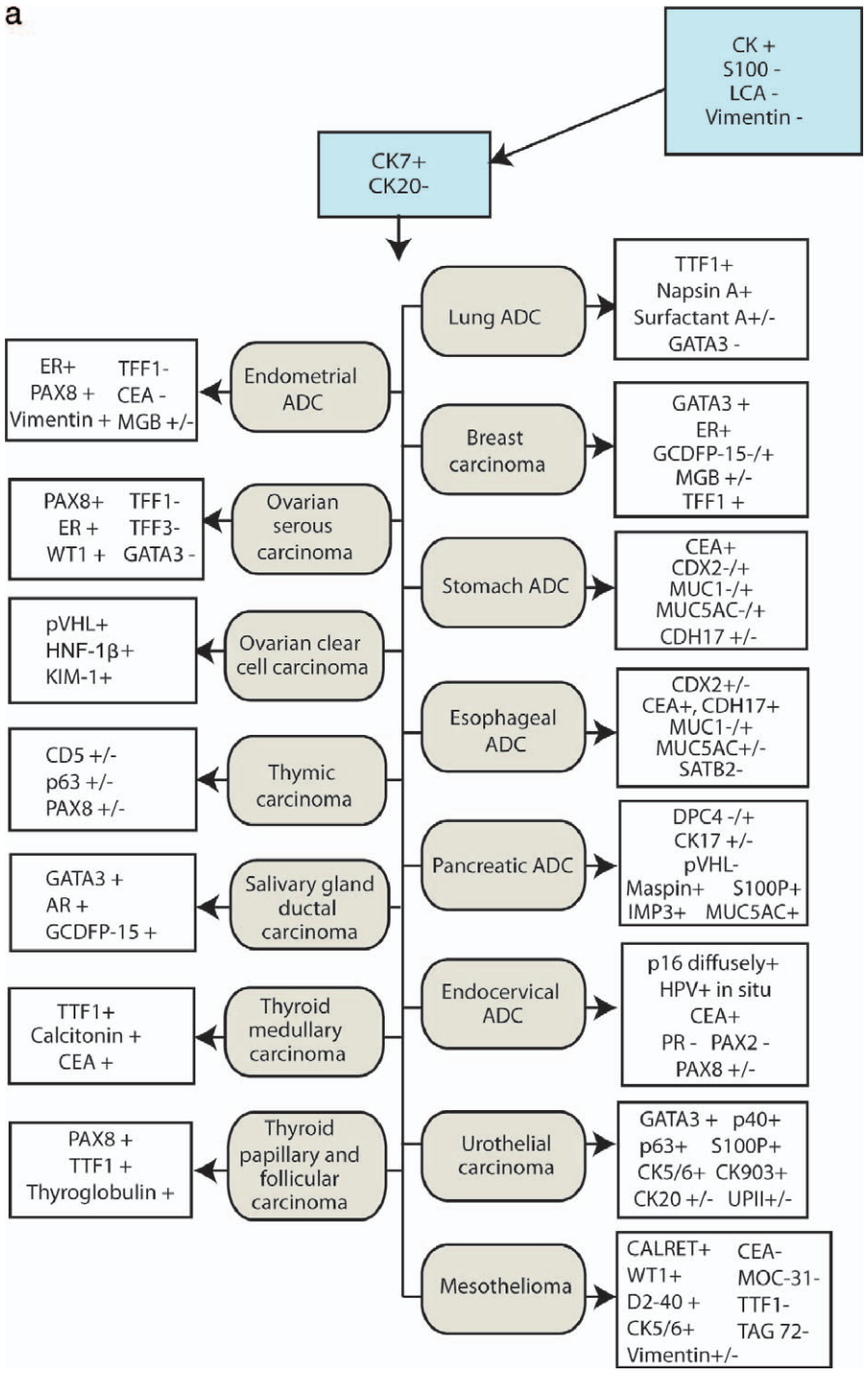

Figure 2. *a through d, The use of CK7 and CK20 to further classify CK⁺ and S100⁻/LCA⁻/vimentin⁻ neoplasms. a, The common entities and useful markers to confirm each diagnosis and the differential diagnosis in CK7⁺/CKC20⁻ carcinomas of uncertain origin. b, The common entities and useful markers to confirm each diagnosis and the differential diagnosis in CK7⁻/CKC20⁺ carcinomas of uncertain origin. * Primary major salivary gland small cell carcinoma, Merkel cell type. ** CK20 perinuclear dot stain. c, The common entities and useful markers to confirm each diagnosis and the differential diagnosis in CK7⁺/CKC20⁺ carcinomas of uncertain origin. d, The common entities and useful markers to confirm each diagnosis and the differential diagnosis in CK7⁻/CKC20⁻ carcinomas of uncertain origin. * Loss of expression of one or more MSI markers (MLH1, MSH2, MSH6, and PMS2). Abbreviations: ACCA, adrenal cortical carcinoma; ADC, adenocarcinoma; AR, androgen receptor; CA-125, cancer antigen 125; CALRET, calretinin; CD5, cluster of differentiation 5; CDH17, cadherin-17; CDX2, caudal type homeobox 2; CEA, carcinoembryonic antigen; chrom, chromogranin; CK, cytokeratin; D2-40, podoplanin; DPC4, SMAD family member 4; ER, estrogen receptor; ERG, ETS-related gene; GATA3, GATA binding protein 3; GCDFP-15, gross cystic disease fluid protein 15; HepPar-1, hepatocyte paraffin-1; HNF-1β, hepatocyte nuclear factor 1β; HPV, human papillomavirus; IMP3, insulin-like growth factor II messenger RNA binding protein-3; INH-A, inhibin-α; KIM-1, kidney injury molecule 1; LCA, leukocyte common antigen; M, membranous; mart-1, melanoma-associated antigen recognized by T-cells 1; maspin, mammary serine protease inhibitor; MCPyV, Merkel cell polyomavirus; MGB, mammaglobin; MLH1, MutL homolog 1; MSH2, MutS protein homolog 2; MSI, microsatellite instability; MUC, mucin; N, nuclear; NANOG, NANOG homeobox; NKX3.1, NK3 homeobox 1; NSE, neuron-specific enolase; OCT4, octamer-binding transcription factor 4; P504S, α-methylacyl-coenzyme A racemase; PAX, paired box gene; pCEA, polyclonal carcinoembryonic antigen; PLAP, placental alkaline phosphatase; PMS2, postmeiotic segregation increased 2; PR, progesterone receptor; PSA, prostate-specific antigen;*

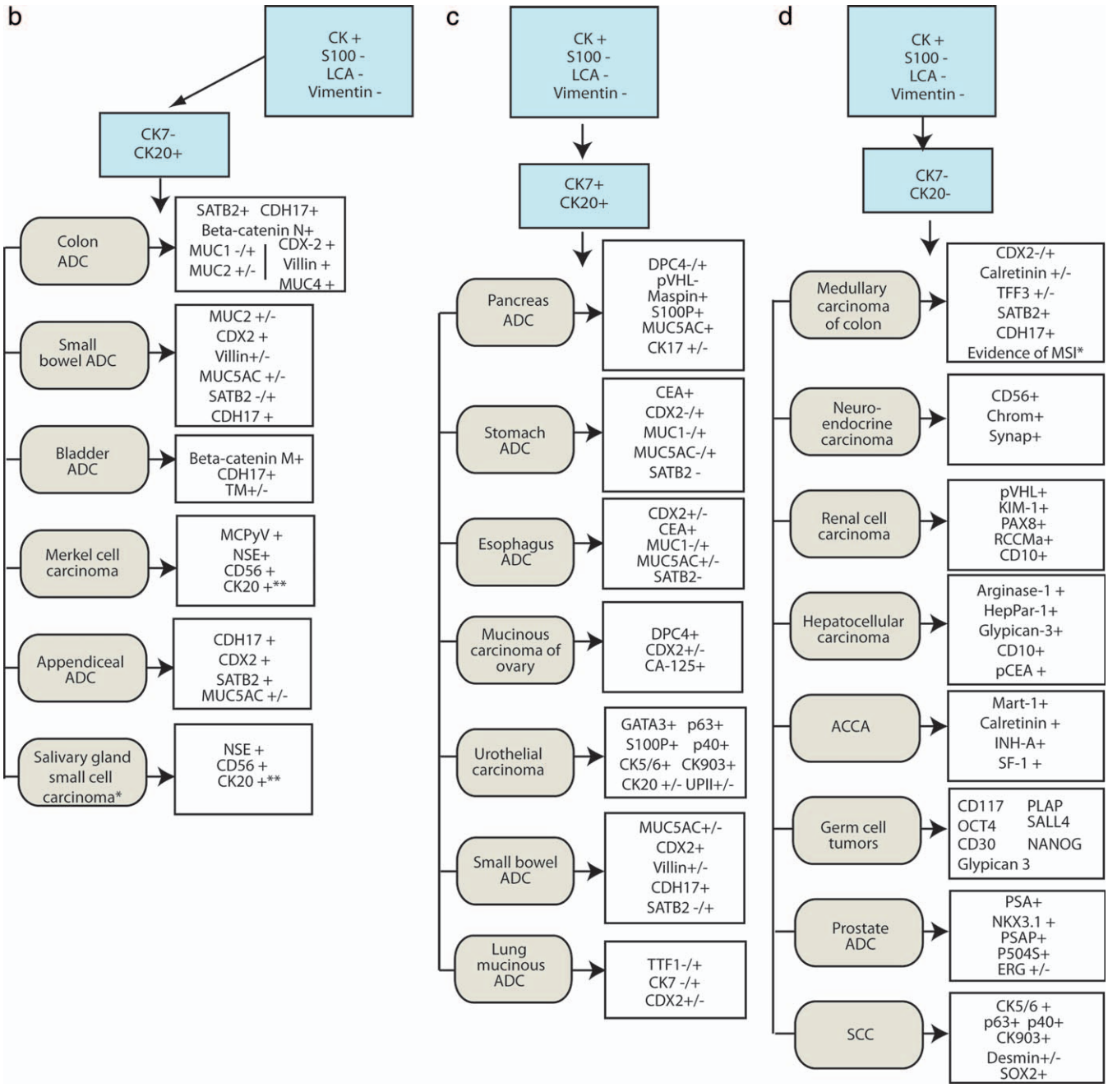

Figure 2, Continued. *PSAP, prostate-specific acid phosphatase; pVHL, von Hippel-Lindau tumor suppressor; RCCma, renal cell carcinoma marker; S100P, placental S100; SALL4, sal-like protein 4; SATB2, special AT-rich binding protein 2; SCC, squamous cell carcinoma; SF-1, steroidogenic factor 1; SOX2, sex-determining region Y box 2; TAG72, tumor-associated glycoprotein 72; synap, synaptophysin; TFF, trefoil factor; TFF3, trefoil factor 3; TM, thrombomodulin; TTF1, thyroid transcription factor 1; UPII, uroplakin II; WT1, Wilms tumor 1. Reprinted with the permission of Springer Science+Business Media from* [2]*Lin F, Prichard JW, Liu H, Wilkerson M, Schuerch C, eds*. Handbook of Practical Immunohistochemistry: Frequently Asked Questions. *New York, NY: Springer.*

Determination of a Broad Category of Neoplasm

A cocktail of AE1/AE3 and CAM 5.2 is an effective panel of markers for identifying an epithelial lineage. The AE1/AE3 stain, by itself, is insufficient to exclude an epithelial lineage. Positive immunoreactivity for AE1/AE3 was reported in 28%, 66%, 48%, and 88% of hepatocellular carcinomas (HCCs), renal cell carcinomas (RCCs), adrenal cortical carcinomas, and pulmonary small cell carcinomas, respectively.[111] Other broad-spectrum cytokeratins containing keratin 8 and keratin 18, such as clones KL1, OSCAR, MAK6, and 5D3/LP3, are also excellent choices as screening cytokeratins.[111] Some carcinomas may show markedly reduced or loss of expression of a broad spectrum of cytokeratins CK7 and CK20, including RCC, HCC, adrenal cortical carcinoma, anaplastic carcinoma, medullary carcinoma of the colon, and metaplastic breast carcinoma. Leukocyte common antigen (LCA), by itself, is insufficient to exclude a potential diagnosis of hematopoietic neoplasm. Some diffuse large B-cell lymphomas, plasmablastic lymphomas, and anaplastic lymphomas can be

Table 2. Tumors That Frequently or Rarely Coexpress Cytokeratin and Vimentin
Carcinomas that frequently express both
Anaplastic thyroid carcinoma
Endometrial carcinoma
Mesothelioma
Metaplastic breast carcinoma
Myoepithelial carcinoma
Renal cell carcinoma
Sarcomatoid carcinoma
Thyroid carcinomas
Mesenchymal tumors that frequently express both
Adamantinoma
Chordoma
DPSRCT
Epithelioid angiosarcoma
Epithelioid sarcoma
Leiomyosarcoma
Malignant rhabdoid tumor
Synovial sarcoma
Carcinomas that rarely express both
Breast carcinoma
GI carcinoma
Lung, non–small cell carcinoma
Ovarian carcinoma
Prostatic carcinoma
Small cell carcinoma

Abbreviations: DPSRCT, desmoplastic small round cell tumor; GI, gastrointestinal.

negative for LCA. A combination of LCA and cluster of differentiation (CD) 43 will cover many lymphomas/myeloid sarcomas.[1] Vimentin is a nonspecific marker; however, a vimentin-negative tumor is unlikely to be a sarcoma (with the exception of alveolar soft part sarcoma), lymphoma, or melanoma. Some carcinomas that frequently coexpress vimentin are summarized in Table 2. A combination of S100 and sex-determining region Y box (SOX) 10 will detect nearly 100% of melanomas and more than 80% of spindle cell/desmoplastic melanomas.[1,112,113] SALL4 and lin-28 homolog A (LIN28) are highly sensitive and specific markers for identifying a tumor of germ cell origin.[88–95,114,115] The markers for determinining many neoplasms are summarized in Table 3.

Tissue-Specific Markers

No single antibody is absolutely sensitive and specific for a particular tumor; however, some antibodies are especially useful when used in small panels.[1–180] The frequently used tissue-specific biomarkers are summarized in Table 1.

Coexpression of Cytokeratin and Vimentin

Follicular, papillary, and medullary thyroid carcinomas stain nearly 100% positive for vimentin. Metaplastic breast carcinoma usually expresses both cytokeratin and vimentin in addition to high–molecular-weight cytokeratins and myoepithelial markers. Alveolar soft part sarcoma is a rare sarcoma that has no immunoreactivity for vimentin. Tumors that express both cytokeratin and vimentin are described in Table 2.

Expression of Epithelial Markers in Nonepithelial Neoplasms

Expression of cytokeratin is not restricted to epithelial neoplasms.[111] Keratin is commonly expressed in some tumors with evidence of epithelial differentiation, such as synovial sarcomas, epithelioid sarcomas, desmoplastic small round cell tumors, chordomas, adamantinoma, and myoepithelial carcinomas. Other mesenchymal tumors can also express cytokeratin, although with a low frequency, including angiosarcomas, epithelioid hemangioendotheliomas, epithelioid leiomyosarcomas, and meningiomas. Aberrant expression of cytokeratin, which tends to be focal, has been reported in other tumors, including undifferentiated pleomorphic sarcomas, rhabdomyosarcomas, malignant rhabdoid tumors and peripheral nerve sheath tumors, clear cell sarcomas, plasmacytomas, diffuse large B-cell lymphomas, anaplastic large cell lymphomas, and melanomas. An example of epithelioid angiosarcoma of the left femur from a 76-year-old woman is shown in Figure 7, A through F. The tumor cells stained positive for cytokeratin, ETS-related gene (ERG) and other vascular markers.

Expression of Hematopoietic Markers in Nonhematopoietic Neoplasms

CD5 has been reported in breast carcinoma, colonic ADC, pancreatic ADC, and lung ADC.[135,137] CD138 is also frequently positive in squamous cell carcinoma (SCC) and can be positive in breast carcinoma, ovarian carcinoma, adrenal cortical carcinoma, and RCC.[1,137]

CD56 is the most sensitive, but not an entirely specific, marker for neuroendocrine neoplasms, including some small cell carcinomas, which may lose expression of cytokeratins and other neuroendocrine markers but still show expression of CD56. A large percentage of thyroid carcinomas are immunoreactive for CD56 as well, as reported in the literature. Our experience showed positivity for CD56 in thyroid tissue and tumor: 100% (20 of 20), 27% (12 of 45), 90% (47 of 52), 44% (16 of 36), and 100% (10 of 10) for normal thyroid follicular cells, papillary thyroid carcinoma, follicular adenoma, follicular carcinoma, and medullary carcinoma, respectively. Hematopoietic markers expressed in nonhematopoietic neoplasms are listed in Table 4.

Review of Selected Antibodies

The following selected antibodies were either recently described or are frequently used in identifying tumors of

Table 3. Markers for Determination of a Variety of Neoplasms						
Marker/Tumor	Carcinoma	Sarcoma	Melanoma	Lymphoma	GCT	Mesothelioma
CK	+	−	−	−	+/−	+
LCA/CD43	−	−	−	+	−	−
S100/SOX10	−/+	−	+	−	−	−
SALL4/LIN28	−	−	−	−	+	−
Vimentin	−/+	+	+	+	−	+/−

Abbreviations: −, <5% of cases are positive; −/+ , <50% of cases are positive; GCT, giant cell tumor; +, >75% of cases are positive; +/−, 50%–75% of cases are positive; CD43, cluster of differentiation 43; CK, a broad spectrum cytokeratin; LCA, leukocyte common antigen; LIN28, lin-28 homolog A; SALL4, sal-like protein 4; SOX10, sex-determining region Y box 10.

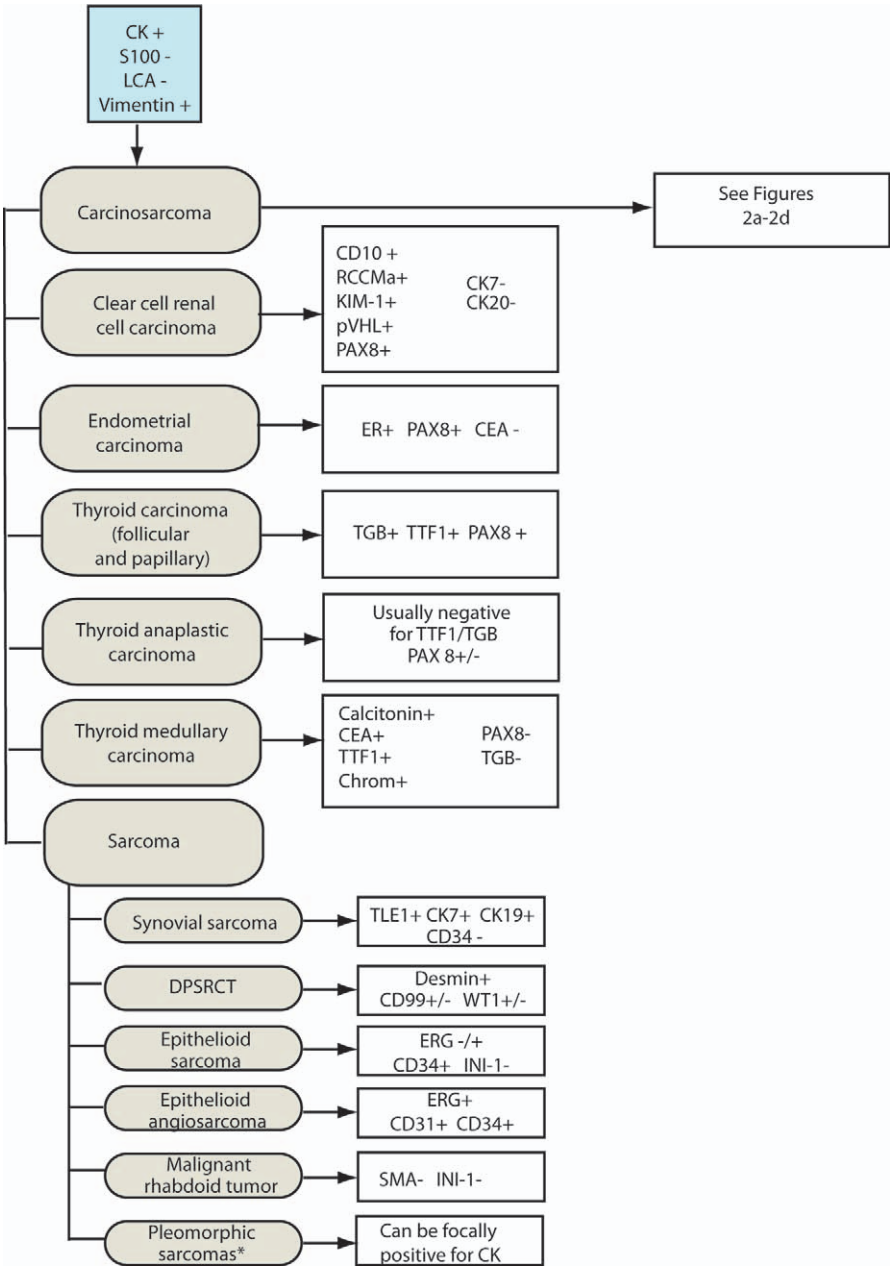

Figure 3. *Neoplasms likely to be CK$^+$/S100$^-$/LCA$^-$/vimentin$^+$ with an outline for performing further workup. Abbreviations: CD, cluster of differentiation; CEA, carcinoembryonic antigen; chrom, chromogranin; CK, cytokeratin; DPSRCT, desmoplastic small round cell tumor; ER, estrogen receptor; ERG, ETS-related gene; INI-1, integrase interactor 1; KIM-1, kidney injury molecule 1; LCA, leukocyte common antigen; PAX8, paired box gene 8; pVHL, von Hippel-Lindau tumor suppressor; RCCma, renal cell carcinoma marker; SMA, smooth muscle actin; TFF1, trefoil factor 1; TGB, thyroglobulin; TLE1, transducin-like enhancer of split 1; TTF1, thyroid transcription factor 1; WT1, Wilms tumor 1. * Pleomorphic leiomyosarcomas, rhabdomyosarcomas, and fibrohistiocytomas. Reprinted with the permission of Springer Science+Business Media from [2]Lin F, Prichard JW, Liu H, Wilkerson M, Schuerch C, eds.* Handbook of Practical Immunohistochemistry: Frequently Asked Questions. *New York, NY: Springer.*

uncertain origin/undifferentiated neoplasms, especially carcinomas. The detailed antibody information is summarized in Table 5. The utility and potential pitfalls of some of these antibodies are discussed in detail.

TTF1.—TTF1 is a homeodomain-containing nuclear transcription protein of the NK2 homeobox (*NKX2*) gene family, which plays a crucial role in organogenesis of the thyroid gland and lung as well as the development of the pituitary gland and the ventral brain.[15] Two commonly used monoclonal antibodies are used in routine practice: clones 8G7G3/1 and SPT24. SPT24 is slightly more sensitive than 8G7G3/1, but its specificity for thyroid and lung ADC appears to be slightly lower. TTF1 is expressed in approximately 75% to 80% of lung ADCs, including nonmucinous bronchioloalveolar carcinomas and signet ring cell type and goblet cell type of primary lung ADCs.[15] In lung, TTF1 was reported[15] to stain positively in 30% of sarcomatoid carcinomas, 40% of large cell carcinomas, 24% of mucinous bronchioloalveolar carcinomas, and 5% of SCCs and to be negative in basaloid SCCs.[15] However, in our experience and that of others, TTF1 (clone 8G7G3/1) was not expressed in pulmonary SCCs.[14] Expression of TTF1 has been reported[15] in pulmonary carcinoid, atypical carcinoid, large cell neuroendocrine carcinomas, and small cell carcinomas in approximately 35%, 50%, 47%, and 90%, respectively. Nearly 100% of thyroid carcinomas, including papillary carcinomas, follicular carcinomas, insular carcinomas, and medullary carcinomas, are positive for TTF1. The staining intensity in medullary thyroid carcinoma tends to be weaker than it is in papillary and follicular carcinomas. In contrast, in our experience, anaplastic thyroid carcinoma tends to be negative. Based on the Ordonez review article,[15] only 13.7% of anaplastic thyroid carcinomas and 33% of Hürthle cell carcinomas were positive for TTF1. TTF1is not entirely specific for lung or thyroid origins. It can be positive in a small percentage of ovarian serous carcinomas,

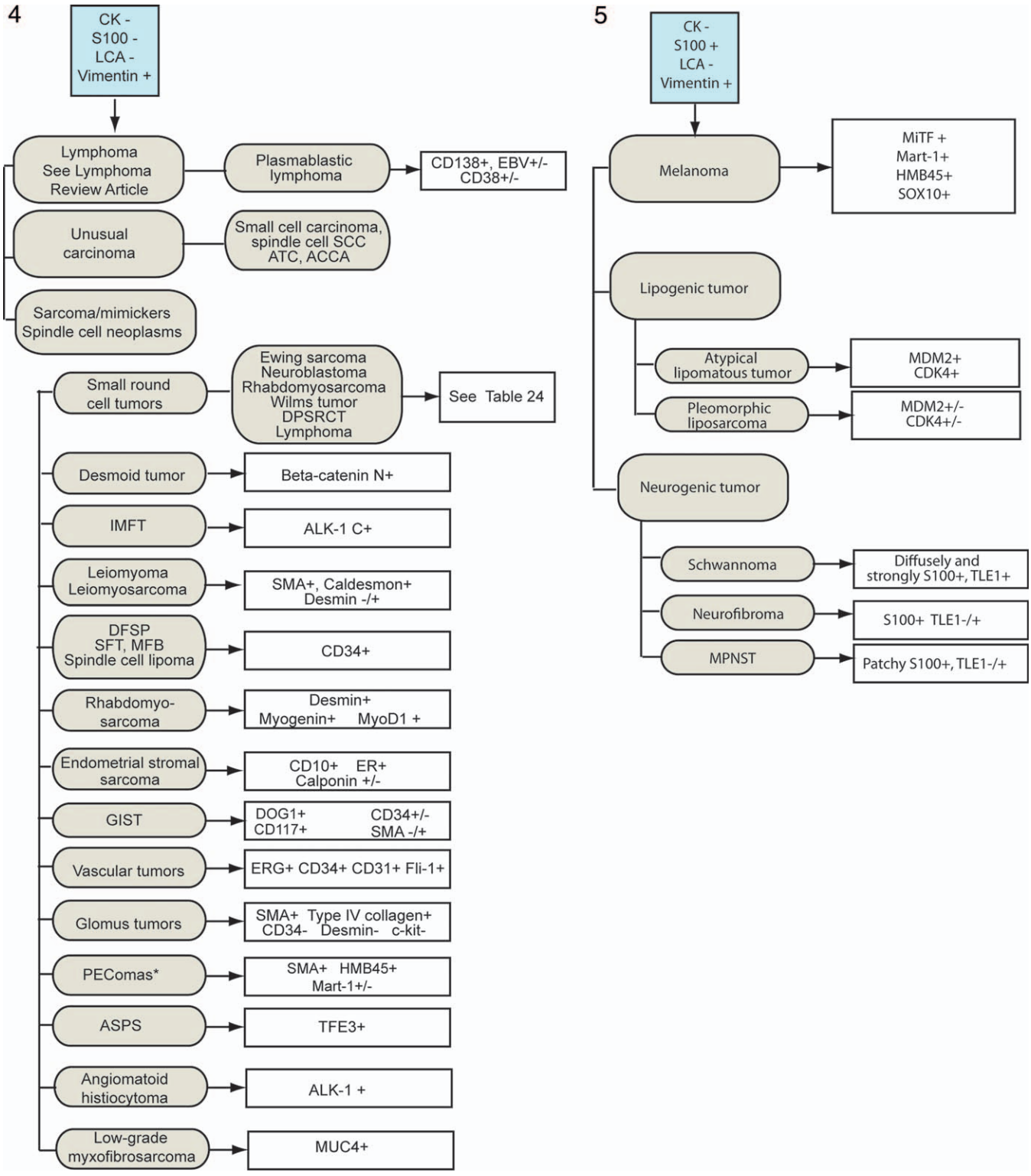

Figure 4. *The neoplasms likely to be CK⁻/S100⁻/LCA⁻/vimentin⁺ with an outline for performing further workup. Abbreviations: ACCA, adrenal cortical carcinoma; ALK-1, anaplastic lymphoma kinase 1; ASPS, alveolar soft part sarcoma; ATC, anaplastic thyroid carcinoma; C, cytoplasmic; CK, cytokeratin; c-kit, kit oncogene; CD, cluster of differentiation; DFSP, dermatofibrosarcoma protuberans; DOG1, discovered on GIST-1; DPSRCT, desmoplastic small round cell tumor; EBV, Epstein-Barr virus; ER, estrogen receptor; ERG, ETS-related gene; Fli-1, friend leukemia virus integration 1; GIST, gastrointestinal stromal tumor; HMB-45, human melanoma black 45; IMFT, inflammatory myofibroblastic tumor; LCA, leukocyte common antigen; Mart-1, melanoma-associated antigen recognized by T cells 1; MFB, myofibroblastoma; MUC4, mucin 4; MyoD1, myogenic differentiation 1; N, nuclear; PEComas, perivascular epithelioid cell tumors; SCC, squamous cell carcinoma; SFT, solitary fibrous tumor; SMA, smooth muscle actin; TFE3, transcription factor E3. * PEComas also include angiomyolipoma, clear cell (sugar) tumor of the lung, and lymphangioleiomyomatosis. S100 can be focally positive in these tumors. Reprinted with the permission of Springer Science+Business Media from* [2]*Lin F, Prichard JW, Liu H, Wilkerson M, Schuerch C, eds.* Handbook of Practical Immunohistochemistry: Frequently Asked Questions. *New York, NY: Springer.*

endometrial ADCs, and endocervical ADCs.[15,181,182] The overall positive rate was significantly lower when using 8G7G3/1 than when using SPT24. Expression of TTF1 in breast carcinoma is exceptionally rare but has been reported.[15] Low-grade neuroendocrine neoplasms/carcinomas of the GI tract and pancreas are usually negative for TTF1. Expression of TTF1 in extrapulmonary small cell carcinomas was reported to be approximately 40%, 60%, 53%, 34%, 18%, 15%, respectively, in the bladder, prostate, GI tract, uterine cervix, colon, and salivary gland.[15] Many polyclonal and monoclonal antibodies to TTF1 are commercially available; however, 8G7G3/1 and SPT24 are the 2 most frequently used. In general, SPT24 stained a higher percentage of extrapulmonary tumors, including glioblastomas and oligodendrogliomas.[15] When using 8G7G3/1, granular cytoplasmic TTF1 staining was observed in normal liver and hepatocellular carcinoma, which was due to the cross-reactivity of the antibody with a 160-kDa cytoplasmic protein.[183]

Napsin A.—Napsin A is an aspartic protease with a molecular weight of 38 kDa, which is expressed in type II pneumocytes and is involved in the N-terminal and C-terminal processing of prosurfactant B in type II pneumocytes. Approximately 75% of pulmonary ADCs express napsin A, including nonmucinous bronchioloalveolar carcinomas. The typical positive staining pattern shows coarse, granular cytoplasmic staining. In contrast, SCCs and small cell carcinomas were generally negative for napsin A.[25] Pereira et al[184] reported napsin A (clone TMU-Ad02; 1:80 dilution) was positive in 25.8%, 7.7%, 37.5%, and 13.3% of SCCs of the lung, skin, penis, and tongue, respectively. Our experience demonstrates TTF1 and napsin A have similar sensitivity and are somewhat complementary to each other in rare instances. In a recent review article,[25] studying 11 published studies, 75% (627 of 836) of the cases of lung ADCs were positive for napsin A, whereas 74.4% (623 of 837) of the cases of lung ADCs were positive for TTF1. Both monoclonal antibodies (TMU-Ad02, IP64, and KCG1.1) and rabbit polyclonal antibodies are available. Approximately 79% of papillary RCCs and 28% of clear cell RCCs were reported to be positive for napsin A.[25] The monoclonal antibody is more specific than the polyclonal antibody is. The polyclonal antibody was positive in a significant percentage of ADCs of the esophagus and thyroid carcinomas. Data from Geisinger Medical Center (Danville, Pennsylvania) on the expression of napsin A are summarized in Table 6.

GATA3.—*GATA3* is 1 of 6 members of a zinc finger transcription factor family, and it is important in promoting and directing cell proliferation, development, and differentiation in many tissues and cell types.[185,186] Together with placental S100 (S100P), it was recently reported as a useful immunohistochemical marker for the detection of urothelial carcinoma and ovarian Brenner tumors.[69,70] Low GATA3 expression has also been suggested to correlate with poor prognosis in breast cancer.[187–190] In our recent immunohistochemical evaluation[191] of GATA3 expression in 1110 carcinomas, 310 cases of normal tissue using tissue microarray (TMA) sections, and 48 breast and bladder biopsy specimens, 62 of 72 urothelial carcinomas (86%) and 138 of 147 breast carcinomas (94%) were positive for GATA3. All others cases, except 2 of 96 endometrial carcinomas (2%), were negative for GATA3. An additional study from our group showed GATA3 expression was seen in 73% of ER$^-$ breast carcinomas. Similar findings have been reported.[77] Based on our study, GATA3 expression was not observed in SCC of the lung.[72] Chang et al[75] reported the same finding. However, the study by Gruver et al[76] reported 23% of pulmonary SCCs can be positive for GATA3. More recent studies demonstrated that GATA3 expression was seen in (1) salivary gland tumors (100% of salivary ductal carcinomas and mammary analogue secretory carcinomas),[74] (2) 80% of metastatic paragangliomas,[73] and (3) 7% of anal SCCs and 19% of uterine cervical SCCs, which tended to be focally positive.[75] Our unpublished data showed approximately 10% of pancreatic ductal adenocarcinomas can be positive for

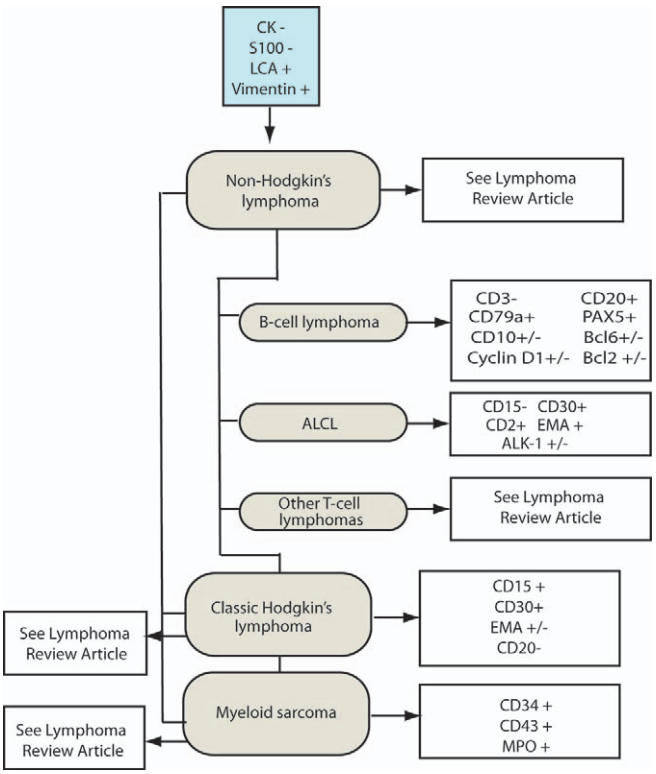

Figure 6. *The neoplasms likely to be CK$^-$/S100$^-$/LCA$^+$/vimentin$^+$ with an outline for performing further workup. Abbreviations: ALCL, anaplastic large cell lymphoma; ALK-1, anaplastic lymphoma kinase 1; Bcl, B-cell CLL/lymphoma; CD, cluster of differentiation; CK, cytokeratin; CLL, chronic lymphocytic leukemia; EMA, epithelial membrane antigen; LCA, leukocyte common antigen; MPO, myeloperoxidase; PAX5, paired box gene 5. Reprinted with the permission of Springer Science+Business Media from [2]Lin F, Prichard JW, Liu H, Wilkerson M, Schuerch C, eds*. Handbook of Practical Immunohistochemistry: Frequently Asked Questions. *New York, NY: Springer.*

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Figure 5. *The neoplasms likely to be CK$^-$/S100$^+$/LCA$^-$/vimentin$^+$ with an outline for performing further workup. Abbreviations: CDK4, cyclin-dependent kinase 4; CK, cytokeratin; HMB-45, human melanoma black 45; Mart-1, melanoma-associated antigen recognized by T cells 1; MDM2, mouse double minute 2 homolog; MiTF, microphthalmia-associated transcription factor; MPNST, malignant peripheral nerve sheath tumor; SOX10, sex-determining region Y box 10; TLE1, transducin-like enhancer of split 1. Reprinted with the permission of Springer Science+Business Media from [2]Lin F, Prichard JW, Liu H, Wilkerson M, Schuerch C, eds*. Handbook of Practical Immunohistochemistry: Frequently Asked Questions. *New York, NY: Springer.*

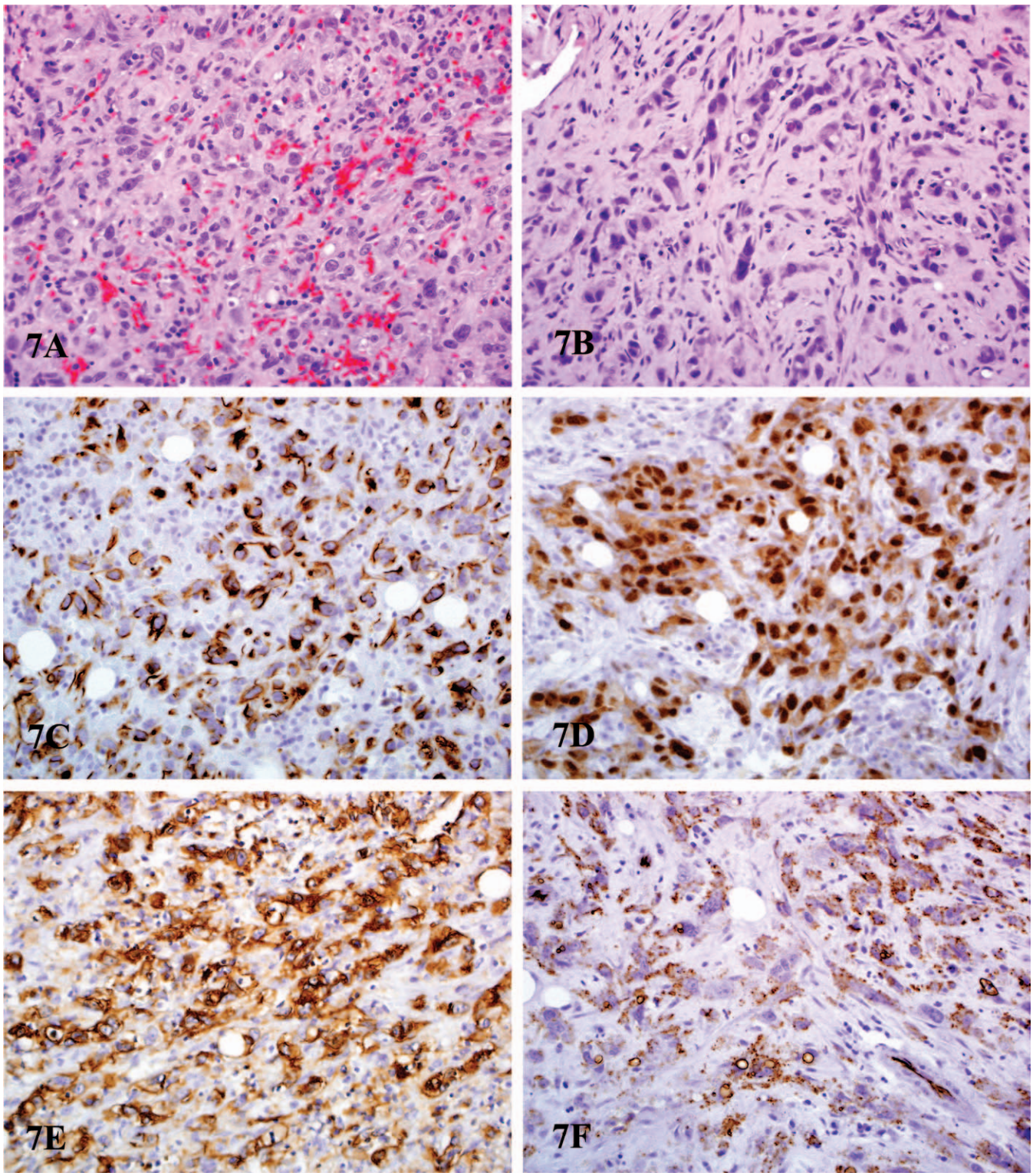

Figure 7. *A through F, An epithelioid angiosarcoma/ hemangioendothelioma from the left femur of a 76-year-old woman. Note that the tumor cells are positive for cytokeratin (AE1/3 and CAM 5.2 [C]), ERG (D), CD31 (E), and CD34 (F). Abbreviations: CD, cluster of differentiation; ERG, ETS-related gene (original magnification ×400 [A through F]).*

GATA3 on tissue microarray sections. In general, GATA3 is a highly sensitive and specific marker for identifying breast and urothelial origin. Figure 8, A through F, illustrates expression of GATA3 in breast carcinoma, urothelial carcinoma, and cervical paraganglioma.

ER, GCDFP-15, and MGB/NY-BR-1.—Estrogen receptor is an important marker when working on tumors of unknown primary, especially in women. The rate of ER$^-$ metastatic breast carcinomas is reported to be in the range of 40% to 52%.[55–62] In addition, ER is not entirely specific for

Table 4. Expression of Hematopoietic Markers in Nonhematopoietic Neoplasms

Marker	Diagnosis
CD5	Thymic carcinoma, cholangiocarcinoma
CD10	RCC, HCC, ESS, choriocarcinoma
CD15	Carcinoma of lung and other organs; renal oncocytoma
CD30	Embryonal carcinoma
CD56	Neuroendocrine carcinomas and thyroid carcinomas
CD138	Carcinoma of lung, cholangiocarcinoma, UC

Abbreviations: CD, cluster of differentiation; ESS, endometrial stromal sarcoma; HCC, hepatocellular carcinoma; RCC, renal cell carcinoma; UC, urothelial carcinoma.

breast primary tumors; it is also positive in most endometrial carcinomas, endocervical ADCs, and ovarian epithelial carcinomas and is infrequently positive in the lung or other primaries.

For many years, ER, GCDFP-15, and MGB have been considered breast-specific immunomarkers in the workup of tumors of uncertain origin.[63–67] However, the sensitivities for GCDFP-15 and MGB were reported to be in the range of 35% to 55% and 65% to 70%, respectively. Our unpublished data on TMA sections of 250 cases of invasive breast carcinomas, including ductal, lobular, and other special types, were even lower: 30% for GCDFP-15 and 50% for MGB, which is similar to reports by Bhargava et al[64] and by Lewis et al.[67] Additionally, MGB was positive in 77% of endometrial ADCs and 31% of endocervical ADCs and other carcinomas as well.[192] Our data showed similar findings.

Table 6. Napsin A Expression in Tumors From Various Organs

Diagnosis	Napsin A, No. (%)
Breast carcinoma, n = 62	0 (0)
Clear cell renal cell carcinoma, n = 53	20 (38)
Colon adenocarcinoma, n = 38	11 (29)
Esophageal adenocarcinoma, n = 30	5 (17)
Lung adenocarcinoma, n = 82	60 (73)
Mesothelioma, n = 23	0 (0)
Pancreatic adenocarcinoma, n = 56	3 (5), focal
Papillary renal cell carcinoma, n = 26	18 (69)
Papillary thyroid carcinoma, n = 45	8 (18)
Squamous cell carcinoma, n = 42	2 (5), focal
Urothelial carcinoma, n = 40	2 (5), focal

NY-BR-1 is a mammary differentiation antigen expressed in normal mammary tissue and its malignant counterpart, with a predominantly cytoplasmic staining pattern. Its expression in breast carcinomas was reported as 58.4% by Woodard et al[86] and 46.6% by Balafoutas et al.[87] However, NY-BR-1 expression is strongly associated with ER expression in both studies. In ER^- breast carcinomas, NY-BR-1 expression was reported to be as low as 18% and 28.4%, respectively. We have tested this antibody (MS-1932-PO, mouse monoclonal antibody; 1:100 dilution; ethylenediaminetetraacetic acid; Thermo Scientific, Waltham, Massachusetts) with several different antigen retrieval and staining conditions; however, the optimal staining condition could not be determined because of the background stain.

TFF1/TFF3.—TFF1 and TFF3 are members of the trefoil family and are characterized by having at least one copy of

Table 5. Summary of Antibody Information

Antibody	Catalog No.	Vendor[a]	Clone	AR/Temp, °C/Time, min	Dilution	pH	Loc
ARG1	5222-1	Epitomics	EPR6672(B)	CC1/95/36	1:500	8	C + N
CAM 5.2	349205	BD Biosciences	CAM 5.2	CC1/95/36	1:4	8	C
CDH17	AC-0095RUO	Epitomics	EP86	CC1/95/36	1:100	8	M
CDX2	235R-16	Cell Marque	EPR2764Y	CC1/95/36	1:600	8	N
CK20	790-4431	Ventana	SP33	CC1/95/64	Predilute	8	C
CK5/6	790-4554	Ventana	D5 + 16B4	CC1/95/64	Predilute	8	C
CK7	307M-95	Cell Marque	OV-TL 12/30	CC1/95/36	1:200	8	C
ER	790-4324	Ventana	SP1	CC1/95/36	Predilute	8	N
GATA3	CM405	Biocare Medical	L50-823	CC1/95/64	1:400	8	N
Glypican-3	261M-98	Cell Marque	1G12	CC1/95/36	Predilute	8	C
LCA	M0701	Dako	2B11 + PD7/26	CC1/95/36	1:80	8	C
Napsin A	AC-0191	Epitomics	EP205	CC1/95/36	1:100	8	C
OCT4	309M-18	Cell Marque	MRQ-10	CC1/95/36	Predilute	8	N
p40	PC373	Millipore	Polyclonal	CC1/95/64	1:2000	8	N
PAX8	CP379AK	Biocare Medical	Polyclonal	CC1/95/36	1:20	8	N
pVHL	SC5575	Santa Cruz	Polyclonal	Protease 1/37/8	1:150	8	M + C
S100	790-2914	Ventana	4C4.9	CC1/95/36	Predilute	8	N + C
SALL4	CM384C	Biocare Medical	6E3	CC1/95/64	1:100	8	N
SATB2	SC-81376	Santa Cruz	SATBA4B10	CC1/95/64	1:20	8	N
TFF1	E100004-RUO	Epitomics	EPR3972	CC1/95/36	1:2000	8	C
TTF1	790-4398	Ventana	8G7G3/1	CC1/95/36	Predilute	8	N
Vimentin	790-2917	Ventana	V9	CC1/95/36	Predilute	8	C
WT1	RB-9267P	Thermo	Polyclonal	CC1/95/36	1:200	8	N

Abbreviations: AR, antigen retrieval; ARG1, arginase-1; C, cytoplasmic; CC1, Cell Conditioning Solution 1 (Ventana); CDH17, cadherin-17; CDX2, caudal type homeobox 2; CK, cytokeratin; ER, estrogen receptor; GATA3, GATA binding protein 3; LCA, leukocyte common antigen; Loc, localization; M, membranous; N, nuclear; OCT4, octamer-binding transcription factor 4; PAX8, paired box gene 8; pVHL, von Hippel-Lindau tumor suppressor; SALL4, sal-like protein 4; SATB2, special AT-rich sequence-binding protein 2; temp, temperature; TFF1, trefoil factor 1; TTF1, thyroid transcription factor 1; WT1, Wilms tumor 1.

[a] Vendor information: BD Biosciences, San Jose, California; Biocare Medical, Concord, California; Cell Marque, Rocklin, California; Dako, Carpinteria, California; Epitomics, Burlingame, California; Millipore, Billerica, Massachusetts; Santa Cruz Biotechnology, Santa Cruz, California; Thermo Scientific, Waltham, Massachusetts; Ventana Medical Systems, Tucson, Arizona.

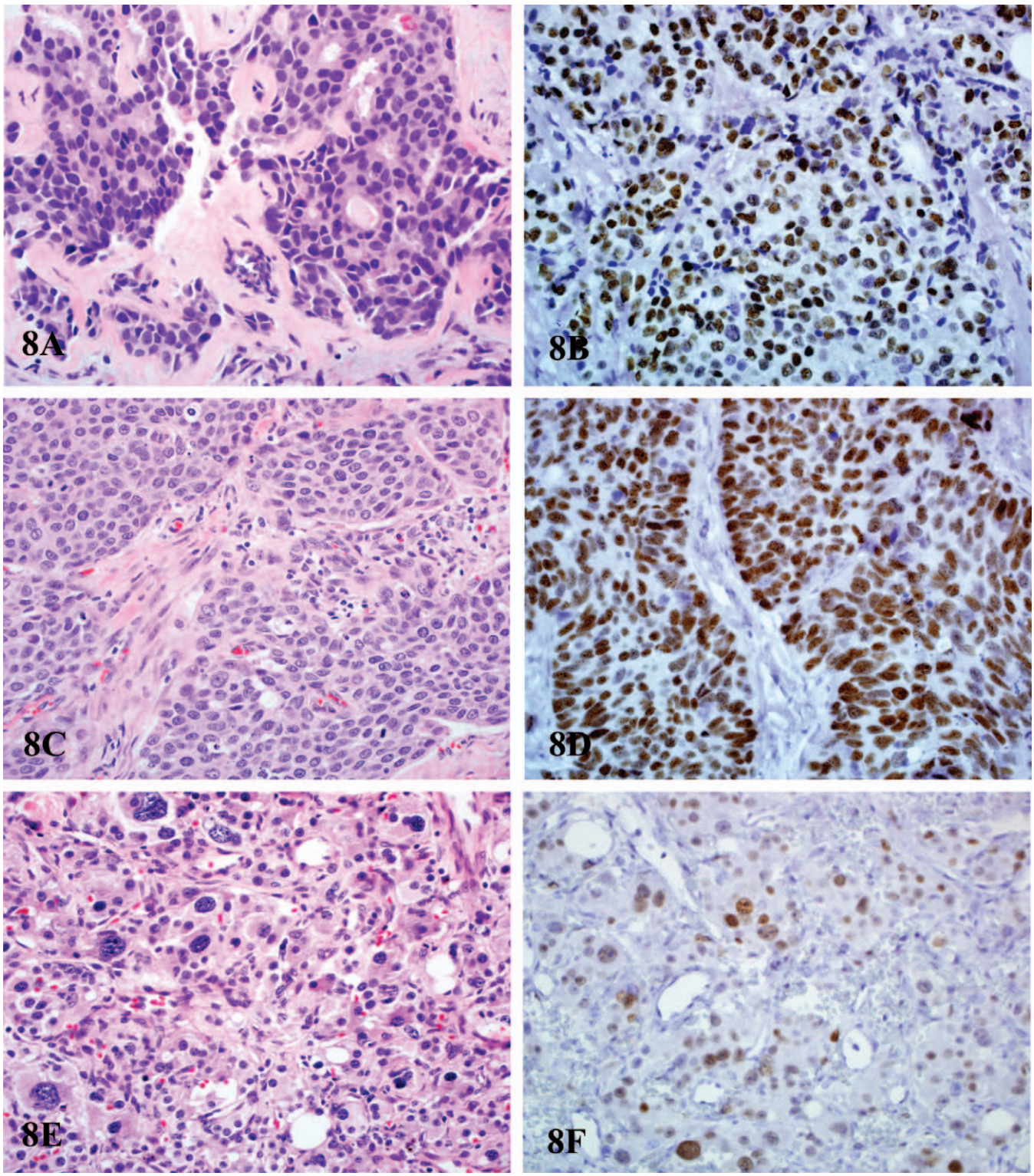

Figure 8. *A through F, Expression of GATA3 in breast carcinoma (A and B), urothelial carcinoma (C and D), and cervical paraganglioma (E and F). Abbreviation: GATA3, GATA binding protein 3 (hematoxylin-eosin, original magnification ×400 [A through F]).*

the trefoil motif, a 40–amino acid domain that contains 3 conserved disulfides.[193] They are stable secretory proteins expressed in GI mucosa.

TFF1.—TFF1 is a protein encoded by the *TFF1* gene, which is also called *pS2* gene. Therefore, TFF1 is also known as protein pS2 or breast cancer estrogen-inducible protein.[194] The expression of TFF1 has been reported to be decreased in carcinomas of the stomach and esophagus when compared with normal mucosa.[193,195] In contrast, TFF1 expression in breast cancer is markedly increased. Our recent studies[83–85] demonstrated that TFF1 was overexpressed in breast carcinomas and colorectal ADCs; under-

Table 7. TFF1 Expression in Carcinomas of Various Organs

Diagnosis	TFF1$^+$ Cases, No. (%)
Breast ductal carcinoma, n = 95	68 (72)
Breast lobular carcinoma, n = 47	41 (87)
Colonic adenocarcinoma, n = 43	39 (91)
Pancreatic adenocarcinoma, n = 50	23 (46)
Endometrial adenocarcinoma, n = 58	4 (7)
Lung adenocarcinoma, n = 111	5 (4.5)
Ovarian serous carcinoma, n = 41	0 (0)
Stomach adenocarcinoma, n = 21	5 (24)
Esophageal adenocarcinoma, n = 30	13 (43)
Prostatic carcinoma, n = 133	50 (37.6)
Other tumors, n = 375	14 (3.7)

Abbreviation: TFF1, trefoil factor 1.

Table 8. TFF3 Expression in Carcinomas From Various Organs

Diagnosis	TFF3$^+$ Cases, No. (%)
Bladder urothelial carcinoma, n = 31	3 (10)
Breast ductal carcinoma, n = 96	81 (84)
Breast lobular carcinoma, n = 48	45 (94)
Colon adenocarcinoma, n = 43	43 (100)
Endometrial carcinoma, n = 38	20 (53)
Esophageal adenocarcinoma, n = 30	17 (57)
Lung adenocarcinoma, n = 111	24 (22)
Ovarian serous carcinoma, n = 41	3 (7)
Pancreas adenocarcinoma, n = 50	20 (40)
Papillary renal cell carcinoma, n = 20	4 (25)
Papillary thyroid carcinoma, n = 47	23 (49)
Prostate carcinoma, n = 97	72 (74)

Abbreviation: TFF3, trefoil factor 3.

expressed in lung ADCs, ovarian carcinomas, and endometrial carcinomas; and was usually absent in germ cell carcinomas, RCCs, and thyroid carcinomas. TFF1 is potentially useful in differentiating breast carcinoma from ADC of the lung, ovary, and endometrium. The results from Geisinger Medical Center are summarized in Table 7.

TFF3.—Functions of TFF3 may include protecting the mucosa from insults, stabilizing the mucus layer, and effecting healing of the epithelium. This gene is a marker of columnar epithelium and is expressed in a variety of tissues, including goblet cells of the intestines and colon. TFF3 overexpression has been observed in carcinomas of the prostate, stomach, and breast. TFF3 is useful in the distinction of breast carcinoma from ovarian serous carcinoma, and its expression is suggestive of intestinal differentiation. Our recent study[196] showed TFF3 expression was retained in poorly differentiated colorectal carcinomas, including 67% of medullary carcinomas of the large intestine. The results from Geisinger Medical Center are summarized in Table 8.

CDX2.—CDX2 is a caudal-related homeobox transcription factor expressed in intestinal epithelium and a highly sensitive immunomarker for GI ADCs, but it may also be expressed in tumors from other organs, such as the pancreas, bile ducts, bladder, uterine cervix, endometrium, and ovary.[30–35] In general, more than 90% of colorectal ADCs and small bowel ADCs are positive for CDX2. However, marked reduction or loss of expression of CK20 and CDX2 was frequently seen in medullary carcinoma of the large intestine.[196–199] Mucinous ADC of the lung is frequently positive for CDX2 and CK20 and negative for TTF1 and napsin A. In addition, sinonasal carcinoma is frequently positive for CDX2 and CK20. Neuroendocrine neoplasms/carcinomas of the ileum, appendix, colon, and rectum are frequently positive for CDX2; in contrast, neuroendocrine tumors (NETs) of the pancreas, lung, and skin are usually negative for CDX2.

SATB2.—SATB2 is a recently described marker that functions as a nuclear matrix-associated transcription factor and an epigenetic regulator.[26,27] Magnusson and coworkers[104] reported that SATB2 in combination with CK20 could identify more than 95% of all colorectal carcinomas. Upper GI carcinomas and pancreatic ADCs are usually negative for SATB2, and ovarian carcinomas and lung ADCs are positive for SATB2 with low frequency.[104] Therefore, SATB2 is a potential marker for identifying a carcinoma of colorectal origin when working on a tumor of unknown primary.[104] Our recent study[196] demonstrated that SATB2 was expressed in 97% of colorectal ADCs (121 of 125), including most of the poorly differentiated colorectal carcinomas and medullary carcinomas of the large intestine, and was rarely positive (3.6%; 60 of 1671 cases) in ADCs from other organs. Another potential utility of SATB2 is to identify neuroendocrine neoplasms/carcinomas of the left colon and rectum because SATB2 is usually negative in other neuroendocrine neoplasms of the GI tract, pancreas, and lung.[200] More recent data[98] show SATB2 is a sensitive marker of osteoblastic differentiation, which, in turn, can be used as a marker to confirm osteosarcoma or a tumor with osteoblastic differentiation.

CDH17.—Cadherin-17 (CDH17), also known as *LI cadherin* (liver-intestine cadherin), is a member of the cadherin superfamily and is a calcium-dependent transmembrane glycoprotein.[99–102] The main function of CDH17 is to mediate cell-cell adhesion and to act as an intestinal peptide transporter.[99–102] CDH17 is expressed in normal glandular epithelium of the GI tract and normal pancreatic ducts.[102] A few reports[101,102] found CDH17 was a highly sensitive marker for GI ADCs and neuroendocrine neoplasms. Panarelli et al[102] also reported that CDH17 was a more-sensitive marker than CDX2 was in identification of GI ADCs and that CDH17 was observed in only a small percentage of ADCs of the lung, breast, ovary, and endometrium. Our recent study[196] demonstrated that CDH17 was expressed in 98% (123 of 125) of the colorectal ADCs, including most of the poorly differentiated colorectal carcinomas and medullary carcinomas of the large intestine and was positive in only 3.3% (55 of 1672) of ADCs from other various organs. In contrast to SATB2, CDH17 was expressed in most neuroendocrine neoplasms/carcinomas of the GI tract and pancreas.[102] The utility of the 4 most frequently used markers for GI differentiation, including SATB2, CDH17, CDX2, and CK20, is summarized in Table 9. A rare example of CK7$^+$ medullary carcinoma of the large intestine with a metastasis to the mesenteric tissue is shown in Figure 9, A through H. The tumor cells were positive for SATB2 and CDH17.

ARG1, Hepatocyte Paraffin-1, and Glypican-3.—ARG1, an enzyme involved in the hydrolysis of arginine to ornithine and urea, was recently recognized in some articles[79–82] as both a sensitive and specific marker for benign and malignant hepatocytes. This is a useful diagnostic marker in the differential diagnosis of HCC versus metastatic tumor and has been studied on both

Table 9. Expression of SATB2, CDH17, CDX2, and CK20 in Gastrointestinal (GI) and Pancreatic Carcinomas				
	Marker			
Diagnosis	SATB2	CDH17	CDX2	CK20
Colon, adenocarcinoma	+	+	+	+
Esophagus, adenocarcinoma	−	+/−	−/+	−/+
ICC	−	+/−	−/+	−/+
Lower GI NET	+	+	+	+
Pancreas, adenocarcinoma	−	−/+	−/+	−/+
Pancreatic NET	−	+/−	−	−
Small bowel, adenocarcinoma	−/+	+	+	+/−
Stomach, adenocarcinoma	−	+/−	−/+	−/+
Upper GI NET	−	+	−	−

Abbreviations: −/+, <50% of cases are positive; −, <5% of cases are positive; +/−, 50%–75% of cases are positive; +, >75% of cases are positive; CDH17, cadherin-17; CDX2, caudal type homeobox 2; CK20, cytokeratin 20; ICC, intrahepatic cholangiocarcinoma; NET, neuroendocrine tumor; SATB2, special AT-rich sequence-binding protein 2.

surgical and cytologic specimens.[79–82] Studies have shown ARG1 is more sensitive and specific than hepatocyte paraffin-1 (HepPar-1) in detecting HCC.[79–82] In our recent study, immunohistochemical evaluation of ARG1, HepPar-1, and glypican-3 expression was performed on 1222 nonhepatic tumors on surgical specimens. The staining results on TMA sections showed that 2.7% (33 of 1222) and 3.1% (38 of 1222) of nonhepatic tumor cases were positive for HepPar-1 and glypican-3, respectively; none (0%, n = 0) were positive for ARG1. In our TMA study, 17 of the 18 HCC cases (94%) were positive for ARG1, which is comparable to the 96% finding of Yan et al,[79] whose findings were similar, with the exception of positive staining in 1 prostatic ADC. Importantly, Yan and coworkers[79] demonstrated that ARG1 has a better sensitivity in identifying higher-grade HCC (moderately differentiated and poorly differentiated HCC; n = 81) than did HepPar-1. That finding is useful because one of the most frequent diagnostic challenges is to differentiate a high-grade HCC from a metastasis, especially on a fine-needle aspiration or small-biopsy specimens. The expression of HepPar-1 in nonhepatocellular tumors is well documented in the literature. For example, Kakar and coworkers[201] reported that the expression of HepPar-1 was observed in 3 of 11 lung ADCs (27%), 6 of 8 esophageal carcinomas (75%), and 7 of 10 gastric carcinomas (70%). Glypican-3 has been shown to be less sensitive and less specific than HepPar-1 and ARG1, with positive staining on various tumors,[201,202] and can occasionally be positive in regenerative nodules associated with cirrhosis.[203]

PAX8/PAX2.—PAX8 is a member of the paired box family of transcription factors involved in the development of thyroid follicular cells and expression of thyroid-specific genes, and, together with PAX2, is involved in regulation of the organogenesis of the kidney and the Müllerian system. PAX8 has been demonstrated to be a highly sensitive and relatively specific marker for thyroid follicular cell tumors, RCCs, ovarian carcinomas, endometrial ADCs, and thymic tumors. Expression of PAX8 in parathyroid tumors and thyroid medullary carcinomas has been reported, but the data were inconsistent.[46] Our limited data showed parathyroid tumors and thyroid medullary carcinomas were rarely positive for PAX8. PAX8 expression has been reported in approximately 70% of anaplastic carcinomas of the thyroid, which has an important clinical implication because most anaplastic thyroid carcinomas are negative for both TTF1 and thyroglobulin.[46] Approximately 50% to 60% of pancreatic NETs and a small percentage of duodenal and rectal NETs were positive for PAX8, which proved to be a consistent staining artifact by the cross-reaction between polyclonal anti-PAX8 antibody and PAX6.[204] Compared with PAX8, PAX2 has a low diagnostic sensitivity for the aforementioned tumors and is negative in parathyroid tumors, thymic tumors, medullary thyroid carcinomas, and pancreatic and GI NETs. It is not entirely certain whether the immunonegativity of PAX2 in those tumors was due to the lack of PAX2 expression or the low sensitivity of the anti-PAX2 antibody that is commercially available now. Both PAX2 and PAX8 are usually positive in nephrogenic adenomas but negative in urothelial carcinomas and prostatic ADCs. Based on the comprehensive review article by Ordonez,[49] the expression of PAX8 versus PAX2 in gynecologic and renal tumors was as follows: serous ovarian carcinoma, 91% versus 48%; endometrial carcinoma, 60% versus 40%; ovarian clear cell carcinoma, 80% versus 43%; clear cell RCC, 94% versus 87%; papillary RCC, 97% versus 60%; chromophobe RCC, 81% versus 16%; and collecting duct carcinoma, 93% versus 38%. We have conducted PAX8 (MRQ-50; Cell Marque; Rocklin, California) immunostaining in 1129 cases of tumors from various organs. The results are summarized in Table 10. Our unpublished data also suggested that PAX2 was negative in endocervical ADCs and positive in all benign endocervical glands and more than 50% of endometrial ADCs.

pVHL.—Inactivation of pVHL on chromosome 3p25-26 by mutation, deletion, or hypermethylation is a frequent event in both hereditary and sporadic clear cell RCCs.[205–207] Genetic alteration of the *VHL* gene appears to be a crucial step in the initiation and progression of clear cell RCC.[207] Our study demonstrated pVHL was expressed in most renal cell neoplasms, including clear cell RCC, papillary RCC, chromophobe RCC, and oncocytoma.[106] More than 90% of metastatic RCCs were positive for pVHL. In addition, 90% of clear cell carcinomas of the ovary and uterus were positive for pVHL.[106] In contrast, many tumors from various organs, including carcinomas of the lung, pancreas, GI tract, adrenal gland, prostate, and bladder, were negative for pVHL. Therefore, pVHL is a highly sensitive and specific marker for identifying metastatic RCC and may serve as a diagnostic marker for clear cell carcinoma of the ovary and uterus.[106] When a metastatic RCC is suspected, a panel of immunomarkers, including PAX8, pVHL, RCC marker (RCCma), and carbonic anhydrase IX (CAIX), should be considered. A single-marker approach is discouraged because (1) PAX8 is

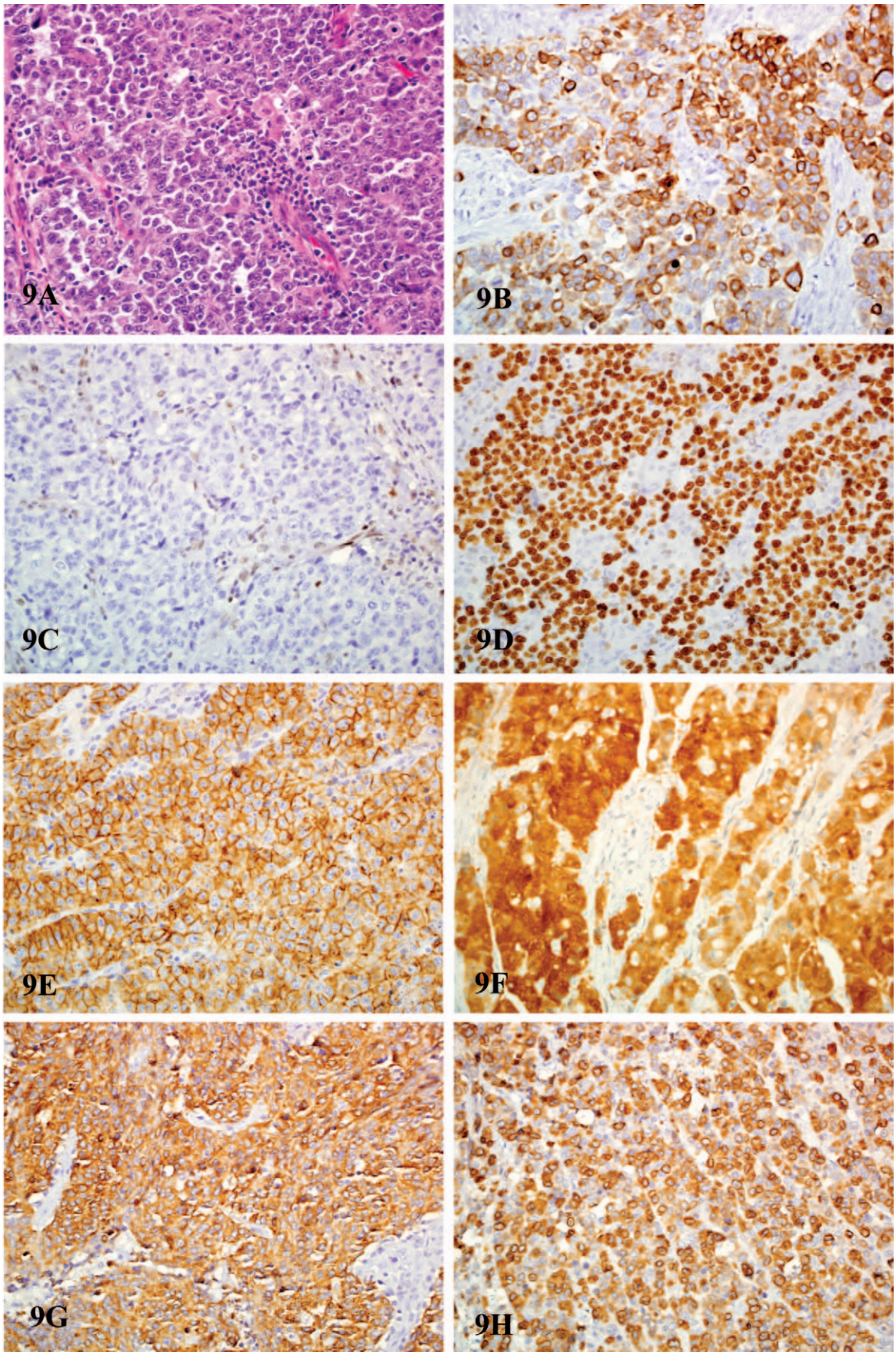

Figure 9. *A through H, A case of CK7^{+} medullary carcinoma of the large intestine with a metastasis to mesenteric tissue on a hematoxylin-eosin–stained section (A), positive for CK7 (B), MLH1 (C), and SATB2 (D), CDH17 (E), calretinin (F), MUC4 (G), and TFF3 (H). Abbreviations: CDH17, cadherin-17; MLH1, MutL homolog 1; MUC4, mucin 4; SATB2, special AT-rich sequence-binding protein 2; TFF3, trefoil factor 3 (original magnification ×400 [A through H]).*

Table 10. Summary of PAX8 Immunostaining Results on 1129 Tumors

Tumor	PAX8$^+$ Cases, No. (%)
Anaplastic thyroid carcinoma, n = 5	2 (40)
Clear cell RCC, n = 36	35 (97)
Colonic adenocarcinoma, n = 68	2 (3)
Endocervical adenocarcinoma, n = 42	22 (53)
Endometrial adenocarcinoma, n = 131	130 (99)
Follicular thyroid carcinoma, n = 36	36 (100)
Medullary thyroid carcinoma, n = 10	0 (0)
Ovarian serous carcinoma, n = 56	56 (100)
Pancreatic NET, n = 32	15 (47)
Papillary RCC, n = 25	25 (100)
Papillary thyroid carcinoma, n = 45	42 (93)
Other tumors,[a] n = 643	0 (0)

Abbreviations: NET, neuroendocrine tumor; PAX8, paired box gene 8; RCC, renal cell carcinoma.

[a] Other tumors: adrenal cortical neoplasm (n = 24), breast ductal carcinoma (n = 110), breast lobular carcinoma (n = 31), cholangiocarcinoma (n = 11), embryonal carcinoma (n = 24), esophageal adenocarcinoma (n = 30), gastric carcinoma (n = 21), hepatocellular carcinoma (n = 18), lung adenocarcinoma (n = 50), lung small cell carcinoma (n = 49), pancreatic carcinoma (n = 50), prostatic carcinoma (n = 100), seminoma (n = 30), urothelial carcinoma (n = 83), and yolk sac tumor (n = 12).

Table 11. SALL4 Immunostaining Results on 1129 Tumors

Tumor	SALL4$^+$ Cases, No. (%)
Colonic adenocarcinoma, n = 68	1 (1)
Embryonal carcinoma, n = 24	24 (100)
Endocervical adenocarcinoma, n = 42	4 (10)
Endometrial carcinoma, n = 131	2 (1.5)
Esophageal adenocarcinoma, n = 30	1 (3)
Ovarian serous carcinoma, n = 56	2 (4)
Seminoma, n = 30	30 (100)
Urothelial carcinoma, n = 83	4 (5)
Yolk sac tumor, n = 12	12 (100)
Other tumors,[a] n = 653	0

Abbreviation: SALL4, sal-like protein 4.

[a] Other tumors include adrenal cortical neoplasm (n = 24), anaplastic thyroid carcinoma (n = 5), breast ductal carcinoma (n = 110), breast lobular carcinoma (n = 31), cholangiocarcinoma (n = 11), clear cell renal cell carcinoma (n = 36), follicular thyroid carcinoma (n = 36), gastric adenocarcinoma (n = 21), hepatocellular carcinoma (n = 18), lung adenocarcinoma (n = 50), lung small cell carcinoma (n = 49), medullary thyroid carcinoma (n = 10), pancreatic adenocarcinoma (n = 50), pancreatic neuroendocrine tumor (n = 32), papillary renal cell carcinoma (n = 25), papillary thyroid carcinoma (n = 45), prostatic adenocarcinoma (n = 100).

also positive in thyroid carcinomas, ovarian, endometrial primaries, and other tumors[46]; (2) RCCma has a low diagnostic sensitivity of approximately 50% for high-grade RCCs and can be positive in other carcinomas, including thyroid carcinomas; and (3) CAIX can be positive in endocervical ADCs and other tumors.[180] The utility of pVHL in the distinction of pancreatic ADC from reactive/benign pancreatic ducts and intrahepatic cholangiocarcinoma is discussed in "Utility of Immunohistochemistry in the Pancreatobiliary Track" (this article is scheduled to appear in the January 2015 issue). Figure 10, A through D, shows a metastatic renal cell carcinoma in bone, which was positive for pVHL, and weakly positive for PAX8 and RCCma.

SALL4, OCT4, and LIN28.—*SALL4*.—A generation of novel germ cell markers, such as SALL4, OCT4, LIN28, NANOG homeobox (NANOG), and SOX2, have been described in the literature in recent years and have proven to be highly sensitive and specific for the differential diagnosis of germ cell tumors when compared with the more traditional germ cell markers, such as placental alkaline phosphatase (PLAP), α-fetoprotein (AFP), CD30, podoplanin (D2-40), CD117, and glypican-3.* SALL4 is a stem cell transcriptional regulator and is an excellent screening marker for germ cell tumors, including seminomas, embryonal carcinomas, yolk sac tumors, and choriocarcinomas, regardless of the origin of the tumor, which can originate from the testis, ovary, mediastinum, central nervous system, and metastatic germ cell tumors.[90–94] The reported sensitivity for seminomas, embryonal carcinomas, and yolk sac tumors is nearly 100%, which is also our experience, as shown in Table 11. Even though SALL4 is a highly specific marker for a broad spectrum of germ cell tumors, its expression has been reported in 89% of hepatoid gastric carcinomas,[95] 58% of embryonal components of hepatoblastoma,[89] 88% of malignant rhabdoid tumors, and 50% of Wilms tumors.[208,209] More recently, a unique punctate/clumped nuclear staining pattern of SALL4 was observed in 30 of 32 HCCs (94%).[88] This staining pattern tended to be focal (<25% of the tumor cells stained) when compared with the diffuse nuclear staining in yolk sac tumors.[88] Additionally, our data showed aberrant SALL4 expression in rare cases of ADC of the colon, esophagus, ovary, endometrium, and endocervix, as shown in Table 11.

OCT4.—OCT4 is another highly sensitive and specific nuclear staining marker for germ cell tumors. Compared with SALL4, OCT4 is negative in yolk sac tumors and expressed in other germ cell tumors.[36–40] A peculiar diffuse cytoplasmic staining of OCT4 has been reported in neuroendocrine tumors and pheochromocytomas,[210,211] which can potentially serve as a neuroendocrine marker. Both SOX2 and NANOG are sensitive nuclear staining markers for embryonal carcinomas and seminomas and are negative in yolk sac tumors.[212]

LIN28.—LIN28 is a recently described RNA-binding protein involved in maintaining the pluripotency of embryonic stem cells. LIN28 is a highly sensitive and relatively specific cytoplasmic marker for primary extragonadal and testicular germ cell tumors, including intratubular germ cell neoplasias, seminomas, yolk sac tumors, and embryonal carcinomas.[114,115] The utility of LIN28 is similar to that of SALL4.

Sex cord stromal tumors are usually negative for these germ cell markers and positive for steroidogenic factor 1 (SF1), inhibin-α, calretinin, and forkhead box L2 (FOXL2).[1,169,213]

RECOMMENDED DIAGNOSTIC IMMUNOHISTOCHEMICAL PANELS

As mentioned earlier, this review article focuses on carcinomas of uncertain origin, which can be separated into 4 main diagnostic groups: (1) CK7$^+$/CK20$^-$, (2) CK7$^+$/CK20$^+$, (3) CK7$^-$/CK20$^+$, and (4) CK7$^-$/CK20$^-$. The common entities within each diagnostic group and the useful markers to confirm each diagnosis and the differential diagnosis are summarized in Figure 2, A through D.

* *References 90–94, 96, 97, 114, 115, 133.*

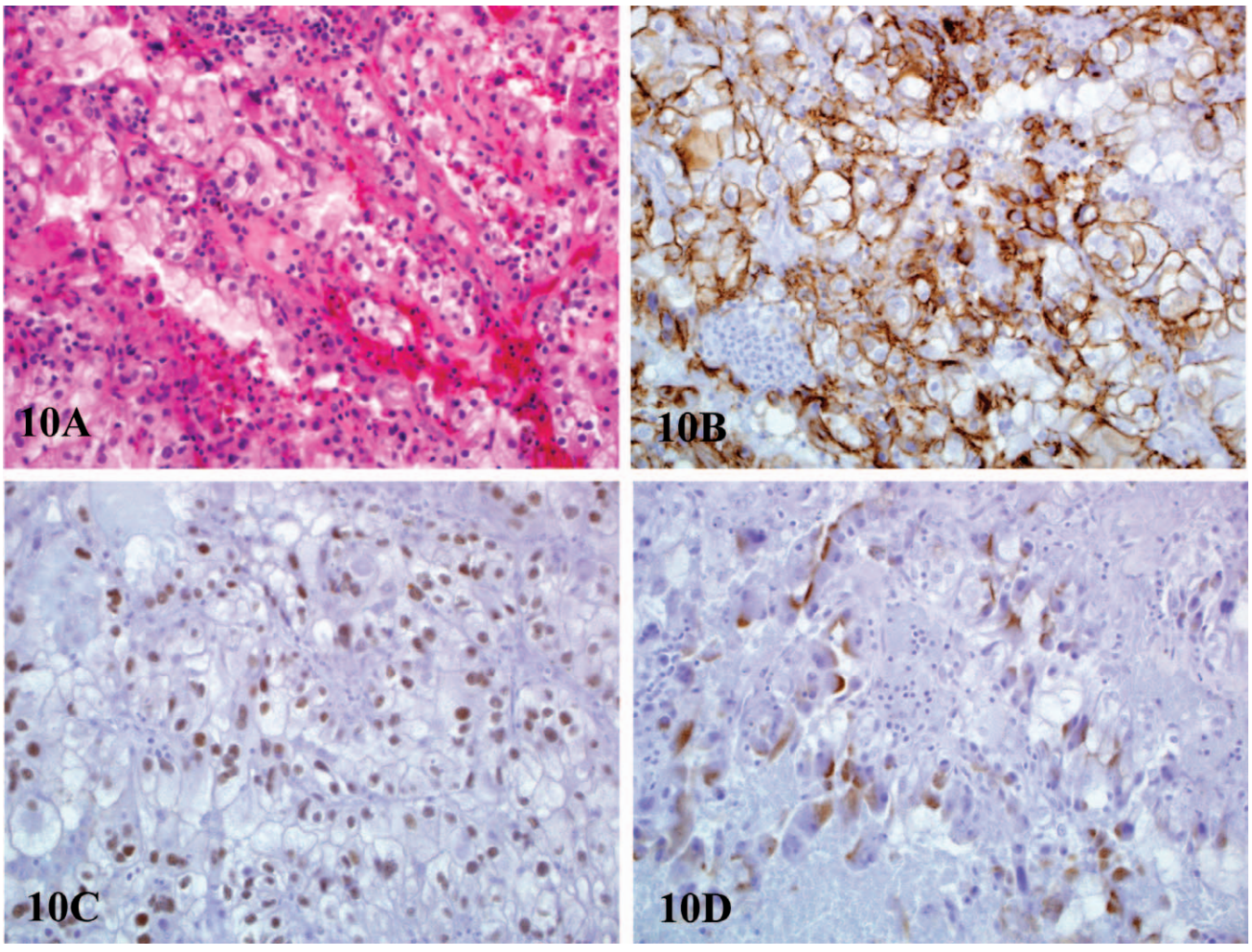

Figure 10. *A through D, A metastatic clear cell renal cell carcinoma (RCC) in bone. Note clear cell RCC on hematoxylin-eosin stain (A), strong pVHL staining (B), nuclear staining for PAX8 (C), and focal cytoplasmic staining for RCCma (D). Abbreviations: PAX8, paired box gene 8; pVHL, von Hippel-Lindau tumor suppressor; RCCma, renal cell carcinoma marker (original magnification ×400 [A through D]).*

Differential Diagnosis of CK7$^+$ and CK7$^+$ and Focal CK20$^+$ Carcinomas

When working on a tumor of an unknown primary, CK7$^+$ or CK7$^+$ and CK20$^+$ carcinomas are nearly always included in the diagnostic consideration. The differential diagnosis usually encompasses a broad spectrum of organs and entities, such as the breast, lung, ovary, uterus, upper GI tract, pancreatobiliary tract, urinary bladder, thyroid, kidney, and mesothelioma. Table 12 summarizes the frequently used markers in the differential diagnosis of those common entities. A significant portion of the CK7$^+$ carcinomas also express ER, and the major differential diagnosis of CK7$^+$/ER$^+$ carcinomas, including tumors from the breast and gynecologic tract, is summarized in Table 13.

In practice, however, each case has a unique presentation; therefore, it is impractical and impossible to discuss here a specific IHC panel for every diagnostically challenging case. However, a few potentially useful IHC panels are recommended in Tables 14 through 19.

In addition to the entities mentioned in Tables 14 through 19, many other entities can present as CK7$^+$ or CK7$^+$ and focally CK20$^+$ carcinomas, including anal/rectal ADCs, ampullary ADCs, common bile duct ADCs, gallbladder ADCs, small-bowel ADCs, renal collecting duct carcinomas, renal medullary carcinomas, medullary thyroid carcinomas, thymic carcinomas, salivary gland carcinomas, ovarian mucinous carcinoma, and SCCs of the uterine cervix.

Differential Diagnosis of CK7$^+$/ER$^+$ Carcinomas

Estrogen receptor is one of the most critical immunomarkers when working on a tumor of uncertain origin or undifferentiated neoplasm, especially in a woman. Estrogen receptor is frequently positive in breast carcinomas and gynecologic primaries. Therefore, ER has a limited role in making the differential diagnosis among those carcinomas. Table 13 includes the most-common ER$^+$ carcinomas when working on a tumor of uncertain origin. GATA3 and TFF1 are 2 recently described, sensitive markers for identifying a tumor with a breast origin, which is rarely positive in other gynecologic carcinomas, including endometrial ADCs, endocervical ADCs, ovarian serous carcinomas, and clear cell carcinomas. TFF1 is expressed in 80% and 90% of breast and colorectal carcinomas, respectively, whereas other carcinomas, including those of the lung, endometrium, and ovary, are rarely positive. Vimentin is expressed in 90% of endometrial ADCs and is negative in other gynecologic

Table 12. Summary of CK7$^+$ and CK7$^+$/CK20$^+$ Epithelial Neoplasms

Marker	LADC	BADC	UGI	PADC	ICC	UC	PRCC	PTC	SADC	MS
Calretinin	−	−	−	−	−	−	−	−	−	+
CDH17	−	−	+/−	+/−	+/−	−	−	−	+	−
CDX2	−	−	−/+	−/+	−/+	−	−	−	+	−
CK20	−	−	−/+	−/+	−/+	+/−	−	−	+/−	−/+
CK5/6	−	−	−	−	−	+	−	−	−	+
CK7	+	+	+	+	+	+	+	+	+	+
ER	−	+	−	−	−	−	−	−	−	−
GATA3	−	+	−	−	−	+	−	−	−	−
Napsin A	+	−	−	−	−	−	+	−	−	−
p40	−	−	−	−	−	+	−	−	−	−
PAX8	−	−	−	−	−	−	+	+	−	−
pVHL	−	−	−	−	+	−	+	−	−	−
RCCma	−	−	−	−	−	−	+	−/+	−	−
TTF1	+	−	−	−	−	−	−	+	−	−
Vimentin	−	−	−	−	−	−	+/−	+	−	+/−

Abbreviations: +, >75% of cases are positive; −, <5% of cases are positive; +/−, 50–75% of cases are positive; −/+, <50% of cases are positive; BADC, breast carcinoma; CDH17, cadherin-17; CDX2, caudal type homeobox 2; CK, cytokeratin; ER, estrogen receptor; GATA3, GATA binding protein 3; ICC, intrahepatic cholangiocarcinoma; LADC, lung adenocarcinoma; MS, mesothelioma; PADC, pancreatic adenocarcinoma; PAX8, paired box gene 8; PRCC, papillary renal cell carcinoma; PTC, papillary thyroid carcinoma; pVHL, von Hippel-Lindau tumor suppressor; RCCma, renal cell carcinoma marker; SADC, small bowel adenocarcinoma; TTF1, thyroid transcription factor 1; UC, urothelial carcinoma; UGI, upper gastrointestinal tract.

carcinomas, with the exception of ovarian endometrioid ADCs, which showed immunoreactivity for vimentin in more than 90% of cases.[214] p16 is a useful marker in distinguishing between endometrial ADC and endocervical ADC; it tends to be diffusely and strongly positive in endocervical ADC (nearly every tumor cell) with only patchy immunoreactivity in endometrial ADC. Human papillomavirus in situ hybridization demonstrated positivity in most endocervical ADCs. pVHL and hepatocyte nuclear factor-1β (HNF-1β) are helpful markers in distinguishing ovarian serous carcinoma from ovarian clear cell carcinoma.[158] Additionally, p53 is usually diffusely and strongly positive or completely negative in serous carcinomas and only focally and weakly positive in ovarian clear cell carcinomas. Kidney injury molecule-1 (KIM-1), which is not currently available commercially, is a sensitive and relatively specific marker for ovarian and uterine clear cell carcinomas;[132] pVHL plays a similar role.[106] No reliable immunomarkers are available for differentiating a uterine serous carcinoma from an ovarian serous carcinoma. Geisinger Medical Center data showed GATA3 was expressed in 92% (90 of 98), 3% (2 of 58), and 0% (0 of 41) of breast, endometrial, and ovarian serous carcinomas, respectively. TFF1 was positive in 77% (109 of 142), 7% (4 of 58), and 0% (0 of 41) of breast, endometrial, and ovarian serous carcinomas, respectively. PAX8 was expressed in 0% (0 of 146), 100% (58 of 58), and 100% (41 of 41) of breast, endometrial, and ovarian serous carcinomas, respectively. WT1 was expressed in 0% (0 of 146), 0% (0 of 58), and 88% (36 of 41) of breast, endometrial, and ovarian serous carcinomas, respectively.

Differential Diagnosis of CK20$^+$/CK7$^-$ Carcinomas

Predominately CK20$^+$ carcinomas include colorectal ADC, small intestinal ADC, bladder ADC, appendiceal ADC, Merkel cell carcinoma, and salivary gland small cell carcinoma. Perinuclear dot-staining patterns are seen in both Merkel cell carcinoma and salivary gland small cell carcinoma. Merkel cell polyomavirus was detected in approximately 80% of Merkel cell carcinomas but not in other high-grade neuroendocrine carcinomas, including salivary gland small cell carcinomas; therefore, Merkel cell polyomavirus is a potentially sensitive and highly specific marker for identification of Merkel cell carcinoma of the skin.[215] CK20$^+$ carcinomas and useful markers are summarized in Table 20.

Differential Diagnosis of CK7$^-$/CK20$^-$ Carcinomas

The following neoplasms usually present as CK7$^-$/CK20$^-$ carcinomas, and some relatively tissue-specific markers may be helpful in reaching a definitive diagnosis. These tumors include, but are not limited to, (1) medullary carcinomas of the colon, (2) some neuroendocrine neoplasms, (3) clear cell

Table 13. Summary of CK7$^+$/ER$^+$ Carcinomas and Useful Markers

Antibody	Breast CA	EMADC	ECADC	OSCA	OCCCA
GATA3	+	−	−	−	−
TFF1	+	−	−	−	−
PAX8	−	+	+/−	+	+/−
WT1	−	−	−	+	−
Vimentin	−	+	−	−	−
p16	−	Patchy +	Diffusely +	+	+/−
HPV in situ	−	−	+/−	−	−
pVHL	−	−	−	−	+/−
HNF-1β	+/−	+/−	+/−	−	+

Abbreviations: +, >75% of cases are positive; −, <5% of cases are positive; +/−, 50%–75% of cases are positive; −/+, <50% of cases are positive; CA, carcinoma; CK, cytokeratin; ECADC, endocervical adenocarcinoma; EMADC, endometrial adenocarcinoma; ER, estrogen receptor; GATA3, GATA binding protein 3; HNF-1β, hepatocyte nuclear factor 1 β; HPV, human papillomavirus; OCCCA, ovarian clear cell carcinoma; OSCA, ovarian serous carcinoma; PAX8, paired box gene 8; pVHL, von Hippel-Lindau tumor suppressor; TFF1, trefoil factor 1; WT1, Wilms tumor 1.

Table 14. Lung Adenocarcinoma Versus Breast Carcinoma

Marker/ Diagnosis	Lung Adenocarcinoma	Breast Carcinoma
ER	−	+
GATA3	−	+
Napsin A	+	−
TFF1	−	+
TTF1	+	−

Abbreviations: +, >75% of cases are positive; −, <5% of cases are positive; ER, estrogen receptor; GATA3, GATA-binding protein 3; TFF1, trefoil factor 1; TTF1, thyroid transcription factor 1.

Table 15. Lung Adenocarcinoma Versus Mesothelioma

Antibody	Lung Adenocarcinoma	Mesothelioma[a]
Calretinin	−	+
CEA	+	−
CK5/6	−	+
D2-40	−	+
MOC-31	+	−
Napsin A	+	−
TTF1	+	−
WT1	−	+

Abbreviations: +, >75% of cases are positive; −, <5% of cases are positive; CEA, carcinoembryonic antigen; CK, cytokeratin; D2-40, podoplanin; MOC-31, epithelial-related antigen clone MOC-31; TTF1, thyroid transcription factor 1; WT1, Wilms tumor 1.

[a] In general, to render a diagnosis of mesothelioma, the tumor should be positive for at least 2 mesothelial markers and negative for 2 carcinoma markers.

Table 16. Lung Versus Upper Gastrointestinal (GI) Tract, Pancreatobiliary Primary, and Urinary Bladder

Markers/ Diagnosis	Lung ADC	Upper GI ADC	Pancreatic ADC	UC
CDH17	−	+/−	−/+	−/+
CDX2	−	−/+	−/+	−
CK20	−	−/+	−/+	+/−
GATA3	−	−	−	+
MUC5AC	−	−/+	+/−	−
Napsin A[a]	+	−/+	−	−
p40	−	−	−	+
TTF1	+	−	−	−

Abbreviations: +, >75% of cases are positive; −, <5% of cases are positive; +/−, 50%–75% of cases are positive; −/+, <50% of cases are positive; ADC, adenocarcinoma; CDH17, cadherin-17; CDX2, caudal type homeobox 2; CK, cytokeratin; GATA3, GATA binding protein 3; MUC5AC, mucin 5AC; TTF1, thyroid transcription factor 1; UC, urothelial carcinoma.

[a] Caution should be taken when using a polyclonal antibody to napsin A. Many esophageal ADCs and some pancreatic ADCs can be positive for napsin A.

RCCs, (4) HCCs, (5) adrenal cortical neoplasm/carcinomas, (6) germ cell tumors, (7) prostatic ADCs, and (8) SCCs. A subset of small cell carcinomas of the lung, gastric ADCs, esophageal ADCs, and mesotheliomas can be $CK7^{-}/CK20^{-}$.

Medullary carcinoma of the colon frequently shows loss of microsatellite instability (MSI) markers, especially MutL homolog 1 (MLH1) and postmeiotic segregation increased 2 (PMS2), and is commonly positive for CDH17, SATB2, calretinin, TFF3, and MUC4.[196–199] CDX2 expression tends to be weak and focal.[196–199] Focal positivity (<25% of the tumor cells stained) for neuroendocrine markers, such as synaptophysin, can be seen.[216]

Table 17. Breast Carcinoma (CA) Versus Upper Gastrointestinal (GI) Tract, Pancreatobiliary Primary, and Urinary Bladder

Markers/ Diagnosis	Breast CA	Upper GI ADC	Pancreatic ADC	UC
CDH17	−	+/−	+/−	−/+
CK20	−	−/+	−/+	+/−
ER	+	−	−	−
GATA3	+	−	−	+
MUC5AC	−	−/+	+/−	−
p40	−	−	−	+
p63	−	−	−	+

Abbreviations: +, >75% of cases are positive; −, <5% of cases are positive; +/−, 50%–75% of cases are positive; −/+, <50% of cases are positive; ADC, adenocarcinoma; CDH17, cadherin-17; ER, estrogen receptor; GATA3, GATA binding protein 3; MUC5AC, mucin 5AC, CK20, cytokeratin 20; UC, urothelial carcinoma.

Table 18. Lung Adenocarcinoma Versus Gynecologic Primaries

Markers/ Diagnosis	Lung ADC	OSC	EMADC	ECADC	OCCC
ER	−	+	+	+/−	+/−
HPV in situ	−	−	−	+	−
Napsin A	+	−	−	−	+/−
PAX8	−	+	+	+	+/−
pVHL	−	−	−	−	+/−
TTF1	+	−	−	−	−
Vimentin	−	−	+	−	−
WT1	−	+	−	−	−

Abbreviations: +, >75% of cases are positive; −, <5% of cases are positive; +/−, 50%–75% of cases are positive; ADC, adenocarcinoma; ECADC, endocervical adenocarcinoma; EMADC, endometrial adenocarcinoma; ER, estrogen receptor; HPV, human papillomavirus; OCCC, ovarian clear cell carcinoma; OSC, ovarian serous carcinoma; PAX 8, paired box gene 8; pVHL, von Hippel-Lindau tumor suppressor; TTF1, thyroid transcription factor 1; WT1, Wilms tumor 1.

Table 19. Papillary Renal Cell Carcinoma (PRCC) Versus Urothelial Carcinoma (UC) Versus Collecting Duct Carcinoma (CDC) Versus Prostatic Adenocarcinoma (PADC)

Markers/ Diagnosis	PRCC	UC	CDC	PADC
CK7	+	+	+	−
GATA3	−	+	−	−
p40	−	+	−	−
P504S	+	−/+	−	+
PAX8	+	−	+	−
PSA	−	−	−	+
RCCma	+	−	+/−	−
S100P	−	+	−	−

Abbreviations: +, >75% of cases are positive; −, <5% of cases are positive; +/−, 50%–75% of cases are positive; −/+, <50% of cases are positive; CK7, cytokeratin 7; GATA3, GATA binding protein 3; P504S, α-methylacyl-CoA racemase; PAX 8, paired box gene 8; PSA, prostate-specific antigen; RCCma, renal cell carcinoma marker; S100P, placental S100.

Neuroendocrine neoplasms/carcinomas are positive for chromogranin, synaptophysin, and CD56. Chromogranin is expressed in well to moderately differentiated neuroendocrine neoplasms/carcinomas and tends to be only focally positive in poorly differentiated neuroendocrine carcinomas/

Table 20. Summary of CK20+ Carcinomas and Useful Markers						
Marker/Diagnosis	CRADC	SADC	BADC	Merkel	APADC	SSCC
β-catenin (nuclear)	+	+/−	−	−	+/−	−
CDH17	+	+	+	−	+	−
CDX2	+	+	+	−	+	−
CK7	−	−/+	−	−	−	−
GATA3	−	−	+/−	−	−	−
SATB2	+	−	N/A	N/A	+	N/A
Synaptophysin	−	−	−	+	−	+

Abbreviations: +, >75% of cases are positive; −, <5% of cases are positive; +/−, 50%–75% of cases are positive; −/+, <50% of cases are positive; APADC, appendiceal adenocarcinoma; BADC, bladder adenocarcinoma; CDH17, cadherin-17; CDX2, caudal type homeobox 2; CK, cytokeratin; CRADC, colorectal adenocarcinoma; GATA3, GATA binding protein 3; N/A, no data available; SADC, small intestinal adenocarcinoma; SATB2, special AT-rich sequence-binding protein 2; SSCC, salivary gland small cell carcinoma.

small cell carcinomas. Caution should be taken when interpreting a diffusely chromogranin-positive tumor as a poorly differentiated neuroendocrine carcinoma/small cell carcinoma. CD56 is a highly sensitive marker for small cell carcinoma; however, its expression is only seen in approximately 50% of pancreatic NETs. Additionally, CD56 can be positive in nonneuroendocrine carcinomas. To differentiate the tissue origin of a given neuroendocrine neoplasm/carcinoma, the following markers are useful and are summarized in Table 21. TTF1 is expressed in approximately 30% of carcinoid tumors, 50% of atypical carcinoid tumors, and 90% of small cell carcinomas of the lung. CDH17 is expressed in NETs of both the upper and lower GI tracts; in contrast, SATB2 is usually expressed in NETs from the left colon and the rectum. Progesterone receptor and PAX8 are expressed in approximately 60% of NETs of the pancreas. Islet-1 has been reported to be a specific marker for well and moderately differentiated NETs of the pancreas and GI tract.[152,153] Pancreatic duodenal homeobox 1 (*PDX1*) is a Hox type transcription factor, which regulates both exocrine and endocrine pancreatic differentiation and maintains the β-cell function. Chan et al[217] reported the expression of PDX1 in 72% (18 of 25) of pancreatic NETs, 10% (3 of 29) of pulmonary NETs, and 4% (1 of 26) GI NETs. The expression of PDX1 was also seen in 5 of 5 metastatic pancreatic NETs (100%) in the liver and 2 of 2 metastatic duodenal NETs (100%) in the liver.[217] They suggested PDX1 was a highly sensitive and specific marker for pancreatic NETs. Another study demonstrated that PDX1 expression was highly associated with the type of hormone secreted by a NET, regardless of whether it was a pancreatic NET or a duodenal NET.[218] Most insulin and gastrin secreting NETs were positive for PDX1, whereas glucagon-positive, somatostatin-positive, or serotonin-positive NETs usually lacked PDX1 expression.[218] Based on those data, PDX1 appears to be a specific marker to identify insulin-positive or gastrin-positive NETs originating either from the pancreas or duodenum. Ileal and appendiceal NETs are usually positive for CK20, CDX2, and CDH17.

Germ cell tumors are frequently negative for CK7 and CK20. SALL4 and LIN28 are excellent screening markers for germ cell tumors, which are positive in nearly 100% of seminomas, embryonal carcinomas, and yolk sac tumors; 70% of choriocarcinomas; and 50% of teratomas.[88–96,114,115] D2-40 and CD117 are specific markers for seminoma; AFP and glypican-3 are specific markers for yolk sac tumors; SOX2, NANOG, and CD30 are relatively specific markers for embryonal carcinomas, although NANOG is also positive in seminomas and SOX2 may be positive in yolk sac tumors; and CD10 and β human chorionic gonadotropin (B-HCG) are specific markers for choriocarcinomas. The useful markers are summarized in Table 22.

More than 90% of clear cell RCCs are negative for both CK7 and CK20. Coexpression of cytokeratin/vimentin is one of the important features for clear cell RCC. Five markers (PAX8, pVHL, RCCma, CD10, and KIM-1) are helpful in confirming the diagnosis of clear cell RCC. PAX8 is likely the most sensitive marker among those 5 markers; however, it also expresses in tumors from the thyroid, gynecologic tract, thymus, and others. RCCma has a low sensitivity of approximately 50% in detecting a high-grade clear cell RCC. Both pVHL and KIM-1 are also expressed in clear cell carcinomas of the uterus and ovary; however, KIM-1 is not commercially available yet. CD10 is a highly sensitive, but not very specific, marker for clear cell RCC. In general, a small panel of antibodies consisting of CAM 5.2, vimentin,

Table 21. Markers for Neuroendocrine Tumors								
Markers/Diagnosis	Lung	Pan	Stoma	Duo	Ileum	Appen	LC	Rec
CDH17	−	+	+	+/−	+	+	+	+
CDX2	−	−	−	−	+	+	+/−	+/−
CK20	−	−	−	−	+	+/−	+	−/+
CK7	+	+/−	+/−	−	−	−	−	−
PAX8	−	+/−	−	−	−	−	−	−/+
PDX1	−	+	−	+	−	−	−	−
PR	−	+/−	−	−	−	−	−	−
SATB2	−	−	−	−	−	−/+	+	+
TTF1	−/+	−	−	−	−	−	−	−

Abbreviations: +, >75% of cases are positive; −, <5% of cases are positive; +/−, 50%–75% of cases are positive; −/+, <50% of cases are positive; appen, appendix; CDH17, cadherin-17; CDX2, caudal type homeobox 2; CK, cytokeratin; duo, duodenum; LC, left colon; pan, pancreas; PAX8, paired box gene 8; PDX1, pancreatic duodenal homeobox 1; PR, progesterone receptor; Rec, rectum; SATB2, special AT-rich sequence-binding protein 2; stoma, stomach; TTF1, thyroid transcription factor 1.

Table 22. Markers for Germ Cell Tumors				
Marker	Seminoma	Embryonal CA	Yolk Sac Tumor	ChorioCA
AFP	−	−	+	−
CD10	−	−	−	+
CD117	+	−	−	−
CD30	−	+	−	−
D2-40	+	−	−	−
Glypican-3	−	−	+	−
LIN28	+	+	+	+/−
NANOG	+	+	−	−
OCT4	+	+	−	+/−
SALL4	+	+	+	+/−
SOX2	−	+	−/+	−
β-HCG	−	−	−	+

Abbreviations: +, >75% of cases are positive; −, <5% of cases are positive; +/−, 50%–75% of cases are positive; −/+, <50% of cases are positive; AFP, α-fetoprotein; CA, carcinoma; CD, cluster of differentiation; chorioCA, choriocarcinoma; D2-40, podoplanin; LIN28, lin-28 homolog; NANOG, NANOG homeobox; OCT4, octamer-binding transcription factor 4; SALL4, sal-like protein 4; SOX2, sex-determining region Y box 2; β-HCG, β-human chorionic gonadotropin.

PAX8, pVHL, and RCCma can serve as an initial panel to confirm a metastatic clear cell RCC. When it comes to a sarcomatoid RCC, an extended panel of antibodies, including cytokeratins, vimentin, PAX8, pVHL, RCCma, CD10, KIM-1, and α-methylacyl-coenzyme A racemase (P504S), is recommended to increase the diagnostic sensitivity.

Most HCCs are negative for CK7 and CK20, with the exception of fibrolamellar HCC, which is usually $CK7^{+}/CK20^{-}$.[219] AE1/AE3 is only positive in approximately 30% of HCCs, whereas more than 90% of HCCs are positive for CAM 5.2, which contains keratin 8.[111] Other cytokeratins, such as 5D3, 5D3/LP34, and KL1 (containing both keratins 8 and 18), are good screening markers for HCC.[111] Many markers are useful for identifying HCC, including ARG1, glypican-3, HepPar-1, CD10, and polyclonal carcinoembryonic antigen (CEA). ARG1 is the most sensitive and specific marker for HCC, including poorly differentiated HCC,[77–82] whereas HepPar-1 is a sensitive, but not very specific, marker for HCC because its immunoreactivity has been reported in many other carcinomas. The diagnostic sensitivity of both ARG1 and HepPar-1 for identifying liver cell origin is more than 90%. Glypican-3 is a good marker for both well-differentiated and poorly differentiated HCC, with a diagnostic sensitivity of approximately 85%.[80] In addition, glypican-3 is not expressed in benign or reactive hepatocytes; in contrast, both ARG1 and HepPar-1 are expressed in both benign and neoplastic hepatocytes. Both CD10 and polyclonal CEA demonstrate a canalicular staining pattern in HCC and benign liver. α-Fetoprotein has limited utility because of its low sensitivity of approximately 25%, but AFP is a highly sensitive marker for hepatoblastoma.

Adrenal cortical neoplasm/carcinomas are epithelial tumors that are usually negative for both CK7 and CK20. Melanoma-associated antigen recognized by T-cells 1 (Mart-1), calretinin, SF-1, and inhibin-α are a group of sensitive and relatively specific markers for identifying adrenal cortical neoplasm/carcinomas. They are usually negative for hepatocellular markers (ARG1, HepPar-1, and glypican-3), and RCC markers (PAX8, RCCma, CD10, CAIX, and pVHL).

More than 90% of prostatic acinar ADCs are negative for CK7 and CK20, with the exception of prostatic ductal ADC, which is usually positive for CK7. Prostate-specific antigen and prostate-specific acid phosphatase are highly sensitive and specific markers for identifying more than 90% of metastatic prostatic ADCs. NK3 homeobox 1 (NKX3.1) is a highly sensitive and specific nuclear staining marker for both primary and metastatic prostatic ADCs and has been reported in nearly 100% of prostatic ADCs.[220,221] An example of metastatic prostatic carcinoma with clear cell features in the pleura is shown in Figure 11, A through D. P504S is another very sensitive, but not totally specific, marker for prostatic ADC. The ERG biomarker was recently described as a specific, but not very sensitive, marker for prostatic ADC, with a diagnostic sensitivity of approximately 40% to 50%. The ERG biomarker is the most sensitive marker for benign and malignant vascular tumors.

Squamous cell carcinomas frequently showed no immunoreactivity for CK7 or CK20. Many markers, such as p40, CK5/6, p63, CK903, SOX2, and desmocollin, are indicative of squamous differentiation. CK5/6 and p40 are the most reliable markers to confirm squamous cell differentiation; however, urothelial carcinomas are also positive for both p40 and CK5/6.

DIAGNOSTIC IMMUNOHISTOCHEMICAL PANELS BASED ON HISTOMORPHOLOGY

If based on the histomorphology alone, 4 major morphologic types of neoplasms are usually encountered, including epithelioid cells (Table 23), small round cells (Table 24), spindle cells (Table 25) and pleomorphic cells (Table 26). Each morphologic category encompasses a wide differential diagnosis. Tables 23 through 26 summarize the useful markers in the differential diagnosis of each category of tumor. An example of metastatic spindle cell melanoma in soft tissue is shown in Figure 12, A through D. The tumor cells are positive for S100, SOX10, microphthalmia-associated transcription factor (MiTF), and Mart-1 but are negative for HMB-45.

NKX2-2 is a transcription factor with a crucial role in differentiation of the central nervous system and pancreatic islets, which has been demonstrated to be expressed in 93% of Ewing sarcomas/primitive neuroectodermal tumors and was not expressed in most of the other small round cell tumors, with exception of olfactory neuroblastomas and a minor subset of small cell carcinomas, synovial sarcomas, mesenchymal chondrosarcomas, and malignant melanomas.[222] NKX2-2 appears to be a more sensitive and specific marker than either CD99 or friend leukemia virus integration 1 (Fli-1) is for Ewing sarcoma/primitive neuroectodermal tumor, based on the limited literature.[222] Additional studies would be needed to validate the diagnostic sensitivity and specificity of this marker.

MOLECULAR PROFILING FOR IDENTIFICATION OF A TUMOR OF UNCERTAIN ORIGIN

The current molecular assays for identifying a tumor of uncertain origin are based on gene expression profiling, by detecting either messenger RNA (mRNA) or microRNA.[223] Cancer Type ID, developed by bioTheranostics (San Diego, California), was based on 92-gene classifier real-time reverse transcription-polymerase chain reaction for detecting mRNA from both frozen tissue sections or formalin-fixed, paraffin-embedded samples, which resulted in an overall diagnostic accuracy of 82% among 39 cancer types.[224,225] In contrast, Rosetta Genomics (Philadelphia,

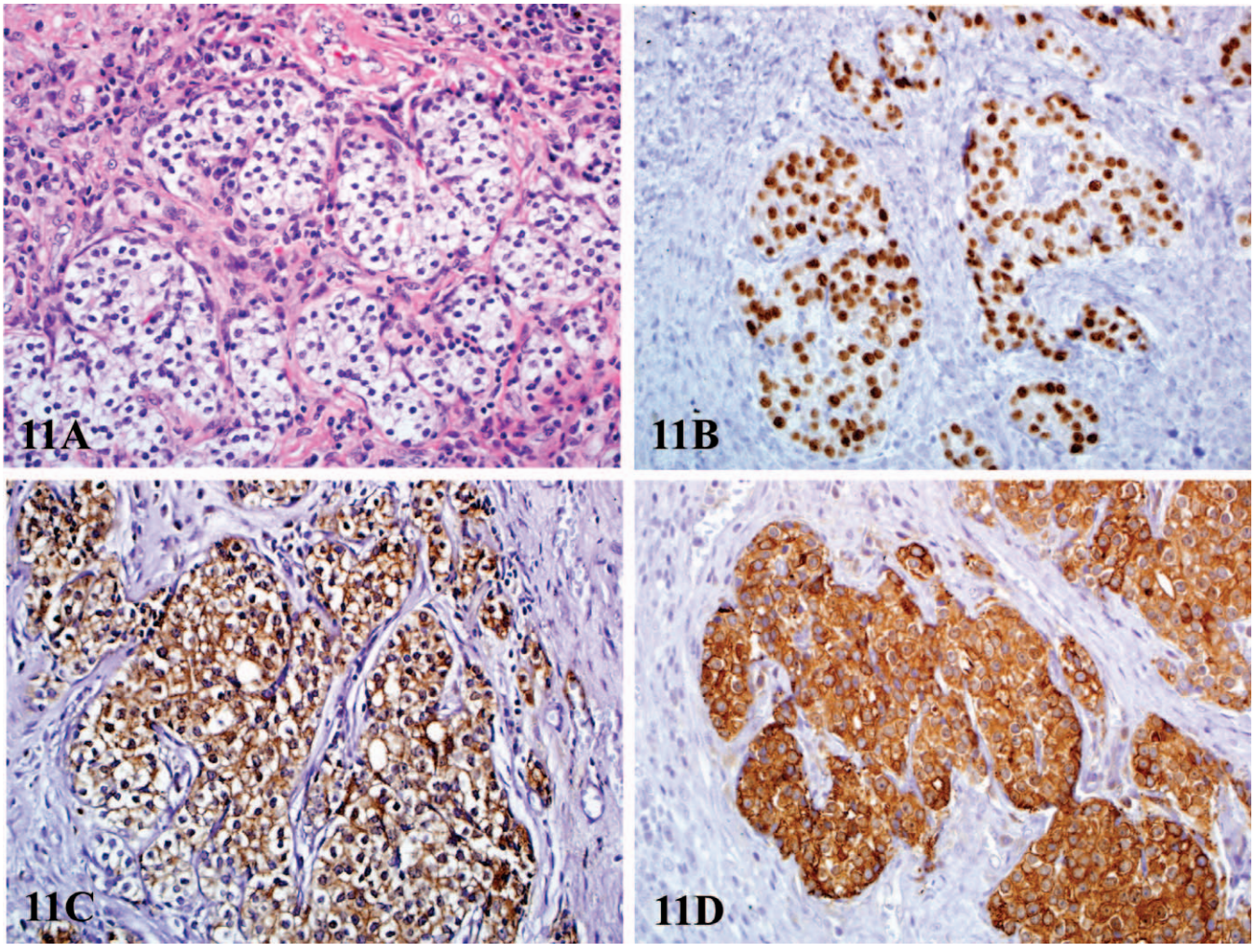

Figure 11. *A through D, A metastatic prostatic adenocarcinoma in the pleura at initial presentation. Note the prostatic adenocarcinoma with clear cell features on hematoxylin-eosin stain (A), nuclear staining for NKX3.1 (B), cytoplasmic PSA staining (C), and cytoplasmic PSAP staining (D). Abbreviations: NKX3.1, NK3 homeobox 1; PSA, prostate-specific antigen; PSAP, prostate-specific acid phosphatase (original magnification ×400 [A through D]).*

Table 23. Markers for Epithelioid Tumors

Diagnosis/Markers	CK	S100	Myo	CD117	SMA	CD34	ERG	Mart-1
Alveolar soft part sarcoma[a]	−	−	−	−	−	−	−	−
Carcinoma	+	−	−	−	−	−	−	−
Chordoma	+	+	−	−	−	−	−	−
Clear cell sarcoma	−	+	−	−	−	−	−	+
Epithelioid angiosarcoma	− or focally +	−	−	−	−	+	+	−
Epithelioid GIST	−	−	−	+	−/+	+/−	−	−
Epithelioid MPNST	− or focally +	Focally +	−	−	−	−	−	−
Epithelioid sarcoma[b]	+	−	−	−	−	+/−	−	−
Melanoma	−	+	−	−/+	−	−	−	+
Mesothelioma	+	−	−	−	−	−	−	−
PEComas[c]	−	−	−	−	+	−	−	+/−

Abbreviations: +, >75% of cases are positive; −, <5% of cases are positive; +/−, 50%–75% of cases are positive; −/+, <50% of cases are positive; CD, cluster of differentiation; CK, cytokeratin (AE1/3$^+$ CAM 5.2); ERG, ETS-related gene; GIST, gastrointestinal stromal tumor; Mart-1, melanoma-associated antigen recognized by T cells 1; MPNST, malignant peripheral nerve sheath tumor; Myo, myogenin; PEComas, perivascular epithelioid cell tumors; SMA, smooth muscle actin.

[a] Alveolar soft part sarcoma may be positive for transcription factor E3 (TFE3), but negative for vimentin.
[b] Epithelioid sarcoma frequently shows loss of integrase interactor 1 (INI-1) expression.
[c] PEComas can be patchy positive for S100 and usually positive for human melanoma black 45 (HMB-45) and melanoma-associated antigen recognized by T-cells 1 (Mart-1) as well.

Table 24. Markers for Small Round Cell Tumors							
Marker/Diagnosis	NB	ES/PNET	RHMS	LYM	DPSRCT	SmCC	PC
CD99	−	+	−/+	+/−	+/−	−	−
CK	−	−	−	−	+	+	−
Desmin	−	−	+	−	+	−	−
Fli-1	−	+	−	+	−	−	−
LCA	−	−	−	+	−	−	−
Myogenin	−	−	+	−	−	−	−
NSE	+	+	−	−	−/+	+/−	−
S100	−	−	−	−	−	−	−
Vimentin	+	+	+	+	+	−	+
WT1	−	−	−	−	+/−	−	−

Abbreviations: +, >75% of cases are positive; −, <5% of cases are positive; +/−, 50%–75% of cases are positive; −/+, <50% of cases are positive; CD, cluster of differentiation; CK, cytokeratin; DPSRCT, desmoplastic small round cell tumor; ES/PNET, Ewing sarcoma/primitive neuroectodermal tumor; Fli-1, friend leukemia virus integration 1; LCA, leukocyte common antigen; LYM, lymphoblastic lymphoma; NB, neuroblastoma; NSE, neuron-specific enolase; PC, plasmacytoma; RHMS, rhabdomyosarcoma; SmCC, small cell carcinoma; WT1, Wilms tumor 1.

Table 25. Markers for Spindle Cell Tumors							
Diagnosis/Markers	CK	S100	Vimentin	SMA	Desmin	CD34	CD117
DFSP	−	−	+	−	−	+	−
Fibrosarcoma	−	−	+	−	−	−	−
GIST	−	−	+	−/+	−	+/−	+
Kaposi sarcoma	−	−	+	−	−	+/−	−
Neurogenic tumor	−	+	+	−	−	−	−
SFT	−	−	+	−	−	+	−
Smooth muscle tumor	−	−	+	+	+	−	−
Spindle cell carcinoma	+	−	+/−	−	−	−	−
Spindle cell melanoma	−	+	+	−	−	−	−
Synovial sarcoma	+	−	+	−	−	−	−

Abbreviations: +, >75% of cases are positive; −, <5% of cases are positive; +/−, 50%–75% of cases are positive; −/+, <50% of cases are positive; CD, cluster of differentiation; CK, cytokeratin; DFSP, dermatofibrosarcoma protuberans; GIST, gastrointestinal stromal tumor; SFT, solitary fibrous tumor; SMA, smooth muscle actin.

Table 26. Markers for Pleomorphic Tumors						
Diagnosis/Markers	CK	Vimentin	S100	Desmin	Myogenin	SMA
Carcinoma	+	−/+	−	−	−	−
Leiomyosarcoma	−	+	−	Focally +	−	+
Liposarcoma	−	+	Focally +	−	−	−
Melanoma	−	+	+	−	−	−
Pleomorphic sarcoma	−	+	−	−	−	−
Rhabdomyosarcoma	−	+	−	+/−	+/−	+/−

Abbreviations: +, >75% of cases are positive; −, <5% of cases are positive; +/−, 50%–75% of cases are positive; −/+, <50% of cases are positive; CK, cytokeratin; SMA, smooth muscle actin.

Pennsylvania) used real-time reverse transcription-polymerase chain reaction to detect microRNA, with a reported accuracy of more than 80%.[226] Pathway Diagnostics (now defunct) also reported high diagnostic accuracy in identification of tumors of uncertain origin using microarray methodology.[227]

CONCLUSIONS

In summary, application of immunohistochemistry in the diagnosis and classification of an undifferentiated neoplasm/tumor of uncertain origin has become an indispensable ancillary study and is crucial in oncologic pathology. The diagnostic accuracy in recognizing the primary site of an undifferentiated neoplasm/tumor of uncertain origin has continuously improved in recent years because of the discovery of additional tissue-specific biomarkers. This is essential in understanding tumor pathobiology, selecting the most effective therapeutic regimen, and predicting a prognostic outcome. This review article (1) highlighted the most important tissue-specific biomarkers with a focus on their application and pitfalls, (2) recommended a step-by-step algorithm for the workup of an undifferentiated neoplasm/tumor of uncertain origin; and (3) identified the most effective IHC panels for solving frequently encountered diagnostic problems and attempting to minimize overuse or underuse of rapidly growing variety of biomarkers. With advances in molecular methodologies, additional tissue-specific biomarkers, specifically for (1) TTF1/napsin A–negative lung ADC, (2) ER/GATA3-negative breast carcinoma, (3) GATA3/S100P/p63-negative urothelial carcinoma, and (4) ADCs of the pancreas/biliary tract, esophagus, and stomach will be discovered in the future. In addition, incorporation of immunohistochemistry and molecular profiling, such as Cancer Type ID, for a highly challenging case or a small biopsy/fine-needle aspiration specimen will further increase diagnostic accuracy in the identification and classification of the primary site of a tumor of uncertain origin/undifferentiated neoplasm.

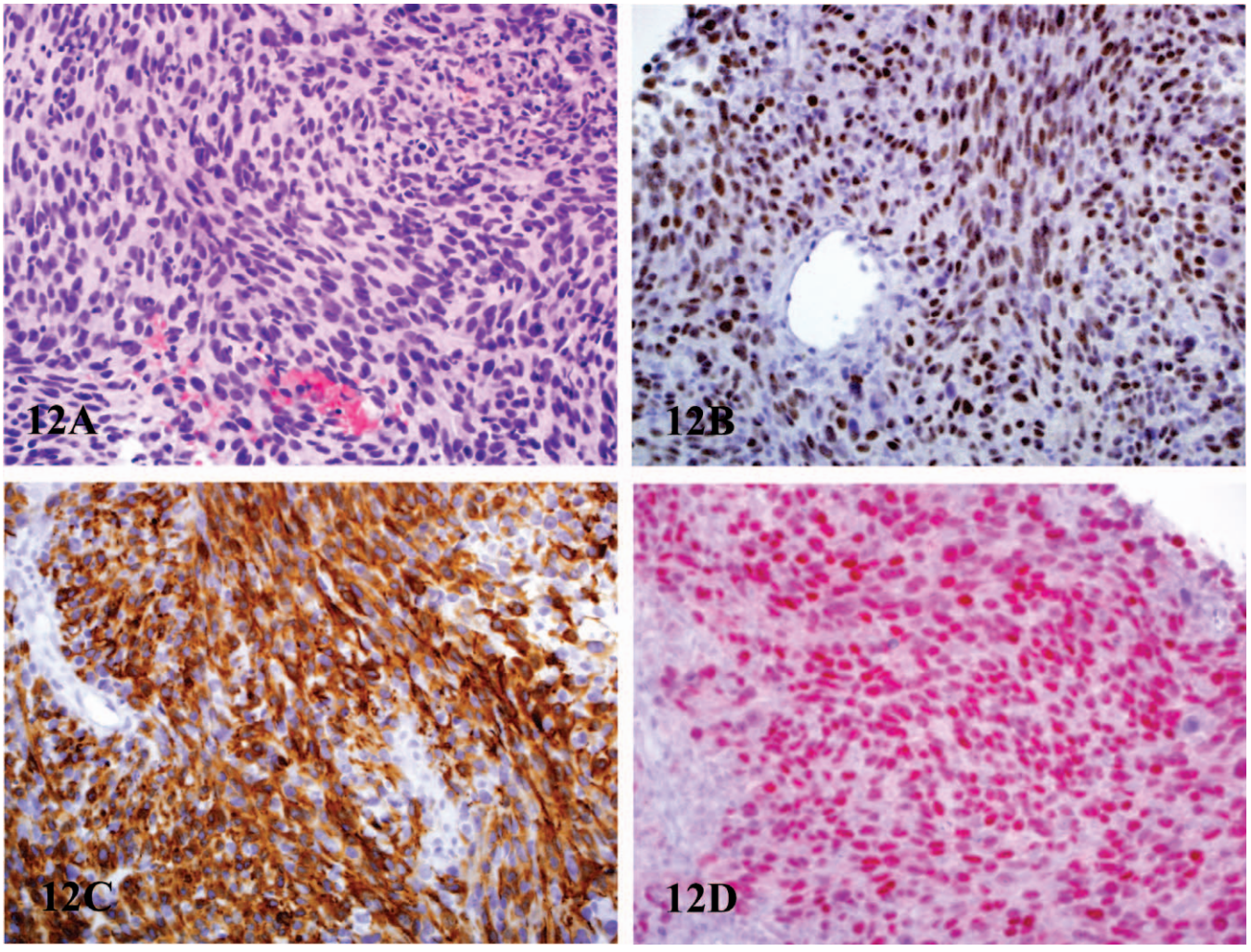

Figure 12. *A through D, A metastatic spindle cell melanoma in soft tissue (A). Note that the tumor cells are positive for SOX10 (B), mart-1 (C), and MiTF (D). The tumor cells are also positive for S100 and vimentin but are negative for HMB-45 (not shown here). Abbreviations: HMB-45, human melanoma black 45; mart-1, melanoma-associated antigen recognized by T-cells 1; MiTF, microphthalmia-associated transcription factor; SOX10, sex-determining region Y box (hematoxylin-eosin, original magnification ×400 [A]; original magnification ×400 [B through D]).*

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References

1. Bahrami A, Truong LD, Ro JY. Undifferentiated tumor: true identity by immunohistochemistry. *Arch Pathol Lab Med*. 2008;132(3):326–348.

2. Lin F, Liu H. Unknown primary/undifferentiated neoplasms in surgical and cytologic specimens. In Lin F, Prichard JW, Liu H, Wilkerson M, Schuerch C., eds. *Handbook of Practical Immunohistochemistry: Frequently Asked Questions*. New York, NY: Springer; 2011:55–83.

3. Dabbs DJ. *Diagnostic Immunohistochemistry*. 3rd ed. Philadelphia, PA: Churchill Livingstone Elsevier; 2010.

4. Taylor C, Cote R, eds. *Immunomicroscopy: A Diagnostic Tool for the Surgical Pathologist. Major Problems in Pathology*. Vol 19. 3rd ed. Philadelphia, PA: Saunders Elsevier; 2006.

5. Chu PG, Weiss LM. *Modern Immunohistochemistry*. New York, NY: Cambridge University Press; 2009.

6. Brown RW, Campagna LB, Dunn JK, Cagle PT. Immunohistochemical identification of tumor markers in metastatic adenocarcinoma: a diagnostic adjunct in the determination of primary site. *Am J Clin Pathol*. 1997;107(1):12–19.

7. DeYoung BR, Wick MR. Immunohistologic evaluation of metastatic carcinomas of unknown origin: an algorithmic approach. *Semin Diagn Pathol*. 2000;17(3):184–193.

8. Varadhachary GR, Abbruzzese JL, Lenzi R. Diagnostic strategies for unknown primary cancer. *Cancer*. 2004;100(9):1776–1785.

9. Dennis JL, Hvidsten TR, Wit EC, et al. Markers of adenocarcinoma characteristic of the site of origin: development of a diagnostic algorithm. *Clin Cancer Res*. 2005;11(10):3766–3772.

10. Park SY, Kim BH, Kim JH, Lee S, Kang GH. Panels of immunohistochemical markers help determine primary sites of metastatic adenocarcinoma. *Arch Pathol Lab Med*. 2007;131(10):1561–1567.

11. Warth A, Muley T, Herpel E, et al. Large-scale comparative analyses of immunomarkers for diagnostic subtyping of non-small-cell lung cancer biopsies. *Histopathology*. 2012;61(6):1017–1025.

12. Krishna M. Diagnosis of metastatic neoplasms: an immunohistochemical approach. *Arch Pathol Lab Med*. 2010;134(2):207–215.

13. Miettinen M. Keratin immunohistochemistry: update of applications and pitfalls. *Pathol Annu*. 1993;28(pt 2):113–143.

14. Ordóñez NG. Thyroid transcription factor-1 is not expressed in squamous cell carcinomas of the lung: an immunohistochemical study with review of the literature. *Appl Immunohistochem Mol Morphol*. 2012;20(6):525–530.

15. Ordóñez NG. Value of thyroid transcription factor-1 immunostaining in tumor diagnosis: a review and update. *Appl Immunohistochem Mol Morphol*. 2012;20(5):429–444.

16. Erickson LA, Papouchado B, Dimashkieh H, Zhang S, Nakamura N, Lloyd RV. Cdx2 as a marker for neuroendocrine tumors of unknown primary sites. *Endocr Pathol*. 2004;15(3):247–252.

17. Chu P, Arber DA. Paraffin-section detection of CD10 in 505 non-hematopoietic neoplasms: frequent expression in renal cell carcinoma and endometrial stromal sarcoma. *Am J Clin Pathol*. 2000;113(3):374–382.

18. Wennerberg AE, Nalesnik MA, Coleman WB. Hepatocyte paraffin 1: a monoclonal antibody that reacts with hepatocytes and can be used for differential diagnosis of hepatic tumors. *Am J Pathol*. 1993;143(4):1050–1054.

19. Chu PG, Ishizawa S, Wu E, Weiss LM. Hepatocyte antigen as a marker of hepatocellular carcinoma: an immunohistochemical comparison to carcinoembryonic antigen, CD10, and alpha-fetoprotein. *Am J Surg Pathol*. 2002;26(8):978–988.

20. Bishop JA, Sharma R, Illei PB. Napsin A and thyroid transcription factor-1 expression in carcinomas of the lung, breast, pancreas, colon, kidney, thyroid, and malignant mesothelioma. *Hum Pathol*. 2010;41(1):20–25.

21. Dejmek A, Naucler P, Smedjeback A, et al. Napsin A (TA02) is a useful alternative to thyroid transcription factor-1 (TTF-1) for the identification of pulmonary adenocarcinoma cells in pleural effusions. *Diagn Cytopathol*. 2007;35(8):493–497.

22. Turner BM, Cagle PT, Sainz IM, Fukuoka J, Shen SS, Jagirdar J. Napsin A, a new marker for lung adenocarcinoma, is complementary and more sensitive and specific than thyroid transcription factor 1 in the differential diagnosis of primary pulmonary carcinoma: evaluation of 1674 cases by tissue microarray. *Arch Pathol Lab Med*. 2012;136(2):163–171.

23. Wu J, Chu PG, Jiang Z, Lau SK. Napsin A expression in primary mucin-producing adenocarcinomas of the lung: an immunohistochemical study. *Am J Clin Pathol*. 2013;139(2):160–166.

24. Kadivar M, Boozari B. Applications and limitations of immunohistochemical expression of "Napsin-A" in distinguishing lung adenocarcinoma from adenocarcinomas of other organs. *Appl Immunohistochem Mol Morphol*. 2013;21(3):191–195.

25. Ordóñez NG. Napsin A expression in lung and kidney neoplasia: a review and update. *Adv Anat Pathol*. 2012;19(1):66–73.

26. Mukhopadhyay S, Katzenstein AL. Comparison of monoclonal napsin A, polyclonal napsin A, and TTF-1 for determining lung origin in metastatic adenocarcinomas. *Am J Clin Pathol*. 2012;138(5):703–711.

27. Xu B, Abourbih S, Sircar K, et al. Diagnostic and prognostic role of immunohistochemical expression of napsin-A aspartic peptidase in clear cell and papillary renal cell carcinoma: a study including 233 primary and metastatic cases [published online ahead of print May 22, 2013]. *Appl Immunohistochem Mol Morphol*. 2014;22(3):206–212. doi:10.1097/PAI.0b013e31828ef24e.

28. Ordóñez NG. Value of PAX8, PAX2, napsin A, carbonic anhydrase IX, and claudin-4 immunostaining in distinguishing pleural epithelioid mesothelioma from metastatic renal cell carcinoma. *Mod Pathol*. 2013;26(8):1132–1143.

29. Chernock RD, El-Mofty SK, Becker N, Lewis JS Jr. Napsin A expression in anaplastic, poorly differentiated, and micropapillary pattern thyroid carcinomas. *Am J Surg Pathol*. 2013;37(8):1215–1222.

30. Barbareschi M, Murer B, Colby TV, et al. CDX-2 homeobox gene expression is a reliable marker of colorectal adenocarcinoma metastases to the lungs. *Am J Surg Pathol*. 2003;27(2):141–149.

31. Werling RW, Yaziji H, Bacchi CE, Gown AM. CDX2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunohistochemical survey of 476 primary and metastatic carcinomas. *Am J Surg Pathol*. 2003;27(3):303–310.

32. Moskaluk CA, Zhang H, Powell SM, Cerilli LA, Hampton GM, Frierson HF Jr. Cdx2 protein expression in normal and malignant human tissues: an immunohistochemical survey using tissue microarrays. *Mod Pathol*. 2003;16(9):913–919.

33. Raspollini MR, Baroni G, Taddei A, Taddei GL. Primary cervical adenocarcinoma with intestinal differentiation and colonic carcinoma metastatic to cervix: an investigation using Cdx-2 and a limited immunohistochemical panel. *Arch Pathol Lab Med*. 2003;127(12):1586–1590.

34. Kaimaktchiev V, Terracciano L, Tornillo L, et al. The homeobox intestinal differentiation factor CDX2 is selectively expressed in gastrointestinal adenocarcinomas. *Mod Pathol*. 2004;17(11):1392–1399.

35. Houghton O, Connolly LE, McCluggage WG. Morules in endometrioid proliferations of the uterus and ovary consistently express the intestinal transcription factor CDX2. *Histopathology*. 2008;53(2):156–165.

36. Jones TD, Ulbright TM, Eble JN, Baldridge LA, Cheng L. OCT4 staining in testicular tumors: a sensitive and specific marker for seminoma and embryonal carcinoma. *Am J Surg Pathol*. 2004;28(7):935–940.

37. Lau SK, Chang KL. OCT4: a sensitive and specific immunohistochemical marker for metastatic germ cell tumors. *Adv Anat Pathol*. 2006;13(2):76–79.

38. Jung SM, Chu PH, Shiu TF, Wu HH, Kuo TT, Chu JJ, Lin PJ. Expression of OCT4 in the primary germ cell tumors and thymoma in the mediastinum. *Appl Immunohistochem Mol Morphol*. 2006;14(3):273–275.

39. Ngan KW, Jung SM, Lee LY, Chuang WY, Yeh CJ, Hsieh YY. Immunohistochemical expression of OCT4 in primary central nervous system germ cell tumours. *J Clin Neurosci*. 2008;15(2):149–152.

40. Sung MT, MacLennan GT, Cheng L. Retroperitoneal seminoma in limited biopsies: morphologic criteria and immunohistochemical findings in 30 cases. *Am J Surg Pathol*. 2006;30(6):766–773.

41. Albadine R, Schultz L, Illei P, et al. PAX8 (+)/p63 (−) immunostaining pattern in renal collecting duct carcinoma (CDC): a useful immunoprofile in the differential diagnosis of CDC versus urothelial carcinoma of upper urinary tract. *Am J Surg Pathol*. 2010;34(7):965–969.

42. Long KB, Srivastava A, Hirsch MS, Hornick JL. PAX8 expression in well-differentiated pancreatic endocrine tumors: correlation with clinicopathologic features and comparison with gastrointestinal and pulmonary carcinoid tumors. *Am J Surg Pathol*. 2010;34(5):723–729.

43. Laury AR, Hornick JL, Perets R, et al. PAX8 reliably distinguishes ovarian serous tumors from malignant mesothelioma. *Am J Surg Pathol*. 2010;34(5):627–635.

44. Sangoi AR, Ohgami RS, Pai RK, Beck AH, McKenney JK, Pai RK. PAX8 expression reliably distinguishes pancreatic well-differentiated neuroendocrine tumors from ileal and pulmonary well-differentiated neuroendocrine tumors and pancreatic acinar cell carcinoma. *Mod Pathol*. 2011;24(3):412–424.

45. Sangoi AR, Karamchandani J, Kim J, Pai RK, McKenney JK. The use of immunohistochemistry in the diagnosis of metastatic clear cell renal cell carcinoma: a review of PAX-8, PAX-2, hKIM-1, RCCma, and CD10. *Adv Anat Pathol*. 2010;17(6):377–393.

46. Ordóñez NG. Value of PAX 8 immunostaining in tumor diagnosis: a review and update. *Adv Anat Pathol*. 2012;19(3):140–151.

47. Chivukula M, Dabbs DJ, O'Connor S, Bhargava R. PAX2: a novel Müllerian marker for serous papillary carcinomas to differentiate from micropapillary breast carcinoma. *Int J Gynecol Pathol*. 2009;28(6):570–578.

48. Ozcan A, Zhai J, Hamilton C, et al. PAX-2 in the diagnosis of primary renal tumors: immunohistochemical comparison with renal cell carcinoma marker antigen and kidney-specific cadherin. *Am J Clin Pathol*. 2009;131(3):393–404.

49. Ordóñez NG. Value of PAX2 immunostaining in tumor diagnosis: a review and update. *Adv Anat Pathol*. 2012;19(6):401–409.

50. Kandil D, Leiman G, Allegretta M, Evans M. Glypican-3 protein expression in primary and metastatic melanoma: a combined immunohistochemistry and immunocytochemistry study. *Cancer*. 2009;117(4):271–278.

51. Maeda D, Ota S, Takazawa Y, et al. Glypican-3 expression in clear cell adenocarcinoma of the ovary. *Mod Pathol*. 2009;22(6):824–832.

52. Wang HL, Anatelli F, Zhai QJ, Adley B, Chuang ST, Yang XJ. Glypican-3 as a useful diagnostic marker that distinguishes hepatocellular carcinoma from benign hepatocellular mass lesions. *Arch Pathol Lab Med*. 2008;132(11):1723–1728.

53. Baumhoer D, Tornillo L, Stadlmann S, Roncalli M, Diamantis EK, Terracciano LM. Glypican 3 expression in human nonneoplastic, preneoplastic, and neoplastic tissues: a tissue microarray analysis of 4,387 tissue samples. *Am J Clin Pathol*. 2008;129(6):899–906.

54. Coston WM, Loera S, Lau SK, et al. Distinction of hepatocellular carcinoma from benign hepatic mimickers using glypican-3 and CD34 immunohistochemistry. *Am J Surg Pathol*. 2008;32(3):433–444.

55. Gomez-Fernandez C, Daneshbod Y, Nassiri M, Milikowski C, Alvarez C, Nadji M. Immunohistochemically determined estrogen receptor phenotype remains stable in recurrent and metastatic breast cancer. *Am J Clin Pathol*. 2008;130(6):879–882.

56. Idirisinghe PK, Thike AA, Cheok PY, et al. Hormone receptor and c-ERBB2 status in distant metastatic and locally recurrent breast cancer. Pathologic correlations and clinical significance. *Am J Clin Pathol*. 2010;133(3):416–429.

57. Broom RJ, Tang PA, Simmons C, et al. Changes in estrogen receptor, progesterone receptor and Her-2/neu status with time: discordance rates between primary and metastatic breast cancer. *Anticancer Res*. 2009;29(5):1557–1562.

58. Finn RS, Press MF, Dering J, et al. Estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2 (HER2), and epidermal growth factor receptor expression and benefit from lapatinib in a randomized trial of paclitaxel with lapatinib or placebo as first-line treatment in HER2-negative or unknown metastatic breast cancer. *J Clin Oncol*. 2009;27(24):3908–3915.

59. Monaco SE, Wu Y, Teot LA, Cai G. Assessment of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status in the fine needle aspirates of metastatic breast carcinomas. *Diagn Cytopathol*. 2013;41(4):308–315.

60. St Romain P, Madan R, Tawfik OW, Damjanov I, Fan F. Organotropism and prognostic marker discordance in distant metastases of breast carcinoma: fact or fiction?: a clinicopathologic analysis. *Hum Pathol*. 2012;43(3):398–404.

61. Aitken SJ, Thomas JS, Langdon SP, Harrison DJ, Faratian D. Quantitative analysis of changes in ER, PR and HER2 expression in primary breast cancer and paired nodal metastases. *Ann Oncol*. 2010;21(6):1254–1261.

62. Chang HJ, Han SW, Oh DY et al. Discordant human epidermal growth factor receptor 2 and hormone receptor status in primary and metastatic breast cancer and response to trastuzumab. *Jpn J Clin Oncol*. 2011;41(5):593–599.

63. Watson MA, Fleming TP. Mammaglobin, a mammary-specific member of the uteroglobin gene family, is overexpressed in human breast cancer. *Cancer Res*. 1996;56(4):860–865.

64. Bhargava R, Beriwal S, Dabbs DJ. Mammaglobin vs GCDFP-15: an immunohistologic validation survey for sensitivity and specificity. *Am J Clin Pathol*. 2007;127(1):103–113.

65. Chia SY, Thike AA, Cheok PY, et al. Utility of mammaglobin and gross cystic disease fluid protein-15 (GCDFP-15) in confirming a breast origin for recurrent tumors. *Breast*. 2010;19(5):355–359.

66. Rollins-Raval M, Chivukula M, Tseng GC, Jukic D, Dabbs DJ. An immunohistochemical panel to differentiate metastatic breast carcinoma to skin from primary sweat gland carcinomas with a review of the literature. *Arch Pathol Lab Med*. 2011;135(8):975–983.

67. Lewis GH, Subhawong AP, Nassar H, et al. Relationship between molecular subtype of invasive breast carcinoma and expression of gross cystic

disease fluid protein 15 and mammaglobin. *Am J Clin Pathol*. 2011;135(4):587–591.

68. Ordóñez NG. The diagnostic utility of immunohistochemistry in distinguishing between epithelioid mesotheliomas and squamous carcinomas of the lung: a comparative study. *Mod Pathol*. 2006;19(3):417–428.

69. Higgins JP, Kaygusuz G, Wang L, et al. Placental S100 (S100P) and GATA3: markers for transitional epithelium and urothelial carcinoma discovered by complementary DNA microarray. *Am J Surg Pathol*. 2007;31(5):673–680.

70. Esheba GE, Longacre TA, Atkins KA, Higgins JP. Expression of the urothelial differentiation markers GATA3 and placental S100 (S100P) in female genital tract transitional cell proliferations. *Am J Surg Pathol*. 2009;33(3):347–353.

71. Gulmann C, Paner GP, Parakh RS, et al. Immunohistochemical profile to distinguish urothelial from squamous differentiation in carcinomas of urothelial tract. *Hum Pathol*. 2013;44(2):164–172.

72. Liu H, Shi J, Wilkerson ML, Lin F. Immunohistochemical evaluation of GATA3 expression in tumors and normal tissues: a useful immunomarker for breast and urothelial carcinomas. *Am J Clin Pathol*. 2012;138(1):57–64.

73. So JS, Epstein JI. GATA3 expression in paragangliomas: a pitfall potentially leading to misdiagnosis of urothelial carcinoma. *Mod Pathol*. 2013;26(10):1365–1370.

74. Schwartz LE, Begum S, Westra WH, Bishop JA. GATA3 immunohistochemical expression in salivary gland. *Head Neck Pathol*. 2013;7(4):311–315.

75. Chang A, Brimo F, Montgomery EA, Epstein JI. Use of PAX8 and GATA3 in diagnosing sarcomatoid renal cell carcinoma and sarcomatoid urothelial carcinoma. *Hum Pathol*. 2013;44(8):1563–1568.

76. Gruver AM, Amin MB, Luthringer DJ, et al. Selective immunohistochemical markers to distinguish between metastatic high-grade urothelial carcinoma and primary poorly differentiated invasive squamous cell carcinoma of the lung. *Arch Pathol Lab Med*. 2012;136(11):1339–1346.

77. Cimino-Mathews A, Subhawong AP, Illei PB, et al. GATA3 expression in breast carcinoma: utility in triple-negative, sarcomatoid, and metastatic carcinomas. *Hum Pathol*. 2013;44(7):1341–1349.

78. Chang A, Amin A, Gabrielson E, et al. Utility of GATA3 immunohistochemistry in differentiating urothelial carcinoma from prostate adenocarcinoma and squamous cell carcinomas of the uterine cervix, anus, and lung. *Am J Surg Pathol*. 2012;36(10):1472–1476.

79. Yan BC, Gong C, Song J, et al. Arginase-1: a new immunohistochemical marker of hepatocytes and hepatocellular neoplasms. *Am J Surg Pathol*. 2010;34(8):1147–1154.

80. Timek DT, Shi J, Liu H, Lin F. Arginase-1, HepPar-1, and glypican-3 are the most effective panel of markers in distinguishing hepatocellular carcinoma from metastatic tumor on fine-needle aspiration specimens. *Am J Clin Pathol*. 2012;138(2):203–210.

81. McKnight R, Nassar A, Cohen C, Siddiqui MT. Arginase-1: a novel immunohistochemical marker of hepatocellular differentiation in fine needle aspiration cytology. *Cancer Cytopathol*. 2012;120(4):223–229.

82. Fujiwara M, Kwok S, Yano H, Pai RK. Arginase-1 is a more sensitive marker of hepatic differentiation than HepPar-1 and glypican-3 in fine-needle aspiration biopsies. *Cancer Cytopathol*. 2012;120(4):230–237.

83. Lin F, Prichard J, Zhang M, Liu H. Identification of an effective immunohistochemical panel in distinction of breast carcinoma from ovarian serous carcinoma [USCAP abstract 1186]. *Mod. Pathol*. 2012;25(S2):283A.

84. Liu H, Yin H, Wang H, Lin F. Re-evaluation of immunohistochemical markers in endometrial adenocarcinomas [USCAP abstract 1189]. *Mod Pathol*. 2012;25(S2):283A.

85. Lin F, Zhu S, Deng H, Liu H. Identification of an effective immunohistochemical panel in distinction of breast carcinoma from lung adenocarcinoma [USCAP abstract 2012]. *Mod. Pathol*. 2012;25(S2):482A.

86. Woodard AH, Yu J, Dabbs DJ, et al. NY-BR-1 and PAX8 immunoreactivity in breast, gynecologic tract, and other $CK7^+$ carcinomas: potential use for determining site of origin. *Am J Clin Pathol*. 2011;136(3):428–435.

87. Balafoutas D, Hausen AZ, Mayer S, et al. Cancer testis antigens and NY-BR-1 expression in primary breast cancer: prognostic and therapeutic implications. *BMC Cancer*. 2013;13:271.

88. Gonzalez-Roibon N, Katz B, Chaux A, et al. Immunohistochemical expression of SALL4 in hepatocellular carcinoma, a potential pitfall in the differential diagnosis of yolk sac tumors. *Hum Pathol*. 2013;44(7):1293–1299.

89. Gnemmi V, Leteurtre E, Sudour-Bonnange H, et al. SALL4 is a marker of the embryonal subtype of hepatoblastoma. *Histopathology*. 2013;63(3):425–428.

90. Cao D, Guo S, Allan RW, Molberg KH, Peng Y. SALL4 is a novel sensitive and specific marker of ovarian primitive germ cell tumors and is particularly useful in distinguishing yolk sac tumor from clear cell carcinoma. *Am J Surg Pathol*. 2009;33(6):894–904.

91. Cao D, Humphrey PA, Allan RW. SALL4 is a novel sensitive and specific marker for metastatic germ cell tumors, with particular utility in detection of metastatic yolk sac tumors. *Cancer*. 2009;115(12):2640–2651.

92. Cao D, Li J, Guo CC, Allan RW, Humphrey PA. SALL4 is a novel diagnostic marker for testicular germ cell tumors. *Am J Surg Pathol*. 2009;33(7):1065–1077.

93. Wang F, Liu A, Peng Y, et al. Diagnostic utility of SALL4 in extragonadal yolk sac tumors: an immunohistochemical study of 59 cases with comparison to placental-like alkaline phosphatase, alpha-fetoprotein, and glypican-3. *Am J Surg Pathol*. 2009;33(10):1529–1539.

94. Mei K, Liu A, Allan RW, et al. Diagnostic utility of SALL4 in primary germ cell tumors of the central nervous system: a study of 77 cases. Mod *Pathol*. 2009;22(12):1628–1636.

95. Ushiku T, Shinozaki A, Shibahara J, et al. SALL4 represents fetal gut differentiation of gastric cancer, and is diagnostically useful in distinguishing hepatoid gastric carcinoma from hepatocellular carcinoma. *Am J Surg Pathol*. 2010;34(4):533–540.

96. Liu A, Cheng L, Du J, et al. Diagnostic utility of novel stem cell markers SALL4, OCT4, NANOG, SOX2, UTF1, and TCL1 in primary mediastinal germ cell tumors. *Am J Surg Pathol*. 2010;34(5):697–706.

97. Rabban JT, Zaloudek CJ. A practical approach to immunohistochemical diagnosis of ovarian germ cell tumours and sex cord-stromal tumours. *Histopathology*. 2013;62(1):71–88.

98. Conner JR, Hornick JL. SATB2 is a novel marker of osteoblastic differentiation in bone and soft tissue tumours. *Histopathology*. 2013;63(1):36–49.

99. Angres B, Kim L, Jung R, Gessner R, Tauber R. LI-cadherin gene expression during mouse intestinal development. *Dev Dyn*. 2001;221(2):182–193.

100. Berndorff D, Gessner R, Kreft B, et al. Liver-intestine cadherin: molecular cloning and characterization of a novel Ca^{2+}-dependent cell adhesion molecule expressed in liver and intestine. *J Cell Biol*. 1994;125(6):1353–1369.

101. Su MC, Yuan RH, Lin CY, Jeng YM. Cadherin-17 is a useful diagnostic marker for adenocarcinomas of the digestive system. *Mod Pathol*. 2008;21(11):1379–1386.

102. Panarelli NC, Yantiss RK, Yeh MM, Liu Y, Chen YT. Tissue-specific cadherin CDH17 is a useful marker of gastrointestinal adenocarcinomas with higher sensitivity than CDX2. *Am J Clin Pathol*. 2012;138(2):211–222.

103. Wang S, Zhou J, Wang XY, et al. Down-regulated expression of SATB2 is associated with metastasis and poor prognosis in colorectal cancer. *J Pathol*. 2009;219(1):114–122.

104. Magnusson K, de Wit M, Brennan DJ, et al. SATB2 in combination with cytokeratin 20 identifies over 95% of all colorectal carcinomas. *Am J Surg Pathol*. 2011;35(7):937–948.

105. Lin F, Shi J, Liu H, et al. Diagnostic utility of S100P and von Hippel-Lindau gene product (pVHL) in pancreatic adenocarcinoma-with implication of their roles in early tumorigenesis. *Am J Surg Pathol*. 2008;32(1):78–91.

106. Lin F, Shi J, Liu H, et al. Immunohistochemical detection of the von Hippel-Lindau gene product (pVHL) in human tissues and tumors: a useful marker for metastatic renal cell carcinoma and clear cell carcinoma of the ovary and uterus. *Am J Clin Pathol*. 2008;129(4):592–605.

107. Liu H, Shi, J, Anandan V, et al. Reevaluation and identification of the best immunohistochemical panel (pVHL, maspin, S100P, IMP-3) for ductal adenocarcinoma of the pancreas. *Arch Pathol Lab Med*. 2012;136(6):601–609.

108. Lok T, Chen L, Lin F, Wang HL. Immunohistochemical distinction between intrahepatic cholangiocarcinoma and pancreatic ductal adenocarcinoma. *Hum Pathol* 2014;45(2):394–400.

109. Sheahan K, O'Keane JC, Abramowitz A, et al. Metastatic adenocarcinoma of an unknown primary site. A comparison of the relative contributions of morphology, minimal essential clinical data and CEA immunostaining status. *Am J Clin Pathol*. 1993;99(6):729–735.

110. Anderson GG, Weiss LM. Determining tissue of origin for metastatic cancers: meta-analysis and literature review of immunohistochemistry performance. *Appl Immunohistochem Mol Morphol*. 2010;18(1):3–8.

111. Ordóñez NG. Broad-spectrum immunohistochemical epithelial markers: a review. *Hum Pathol*. 2013;44(7):1195–1215.

112. Ordóñez NG. Value of SOX10 immunostaining in tumor diagnosis. *Adv Anat Pathol*. 2013;20(4):275–283.

113. Ordóñez NG. Value of melanocytic-associated immunohistochemical markers in the diagnosis of malignant melanoma: a review and update. *Hum Pathol*. 2014;45(2):191–205.

114. Cao D, Liu A, Wang F, et al. RNA-binding protein LIN28 is a marker for primary extragonadal germ cell tumors: an immunohistochemical study of 131 cases. *Mod Pathol*. 2011;24(2):288–296.

115. Cao D, Allan RW, Cheng L, et al. RNA-binding protein LIN28 is a marker for testicular germ cell tumors. *Hum Pathol*. 2011;42(5):710–718.

116. Herawi M, Drew PA, Pan CC, Epstein JI. Clear cell adenocarcinoma of the bladder and urethra: cases diffusely mimicking nephrogenic adenoma. *Hum Pathol*. 2010;41(4):594–601.

117. Miettinen M. Keratin 20: immunohistochemical marker for gastrointestinal, urothelial, and Merkel cell carcinomas. *Mod Pathol*. 1995;8(4):384–388.

118. Chu PG, Weiss LM. Expression of cytokeratin 5/6 in epithelial neoplasms: an immunohistochemical study of 509 cases. *Mod Pathol*. 2002;15(1):6–10.

119. Armah HB, Parwani AV. Xp11.2 translocation renal cell carcinoma. *Arch Pathol Lab Med*. 2010;134(1):124–129.

120. Hornick JL, Dal Cin P, Fletcher CD. Loss of INI1 expression is characteristic of both conventional and proximal-type epithelioid sarcoma. *Am J Surg Pathol*. 2009;33(4):542–550.

121. Cheng JX, Tretiakova M, Gong C, Mandal S, Krausz T, Taxy JB. Renal medullary carcinoma: rhabdoid features and the absence of INI1 expression as markers of aggressive behavior. *Mod Pathol*. 2008;21(6):647–652.

122. Parker DC, Folpe AL, Bell J, et al. Potential utility of uroplakin III, thrombomodulin, high molecular weight cytokeratin, and cytokeratin 20 in noninvasive, invasive, and metastatic urothelial (transitional cell) carcinomas. *Am J Surg Pathol*. 2003;27(1):1–10.

123. Miettinen M, Sarlomo-Rikala M. Expression of calretinin, thrombomodulin, keratin 5, and mesothelin in lung carcinomas of different types: an immunohistochemical analysis of 596 tumors in comparison with epithelioid mesotheliomas of the pleura. *Am J Surg Pathol*. 2003;27(2):150–158.

124. Miettinen M, Franssila KO. Variable expression of keratins and nearly uniform lack of thyroid transcription factor 1 in thyroid anaplastic carcinoma. *Hum Pathol*. 2000;31(9):1139–1145.

125. Wang HL, Lu DW, Yerian LM, et al. Immunohistochemical distinction between primary adenocarcinoma of the bladder and secondary colorectal adenocarcinoma. *Am J Surg Pathol*. 2001;25(11):1380–1387.

126. Hishima T, Fukayama M, Fujisawa M, et al. CD5 expression in thymic carcinoma. *Am J Pathol*. 1994;145(2):268–275.

127. Binh MB, Sastre-Garau X, Guillou L, et al. MDM2 and CDK4 immunostainings are useful adjuncts in diagnosing well-differentiated and dedifferentiated liposarcoma subtypes: a comparative analysis of 559 soft tissue neoplasms with genetic data. *Am J Surg Pathol*. 2005;29(10):1340–1347.

128. Kumar S, Perlman E, Harris CA, Raffeld M, Tsokos M. Myogenin is a specific marker for rhabdomyosarcoma: an immunohistochemical study in paraffin-embedded tissues. *Mod Pathol*. 2000;13(9):988–993.

129. Pallesen G, Hamilton-Dutoit SJ. Ki-1 (CD30) antigen is regularly expressed by tumor cells of embryonal carcinoma. *Am J Pathol*. 1988;133(3):446–450.

130. Jiang Z, Fanger GR, Woda BA, et al. Expression of α-methylacyl-CoA racemase (p504s) in various malignant neoplasms and normal tissues: a study of 761 cases. *Hum Pathol*. 2003;34(8):792–796.

131. Tretiakova MS, Sahoo S, Takahashi M, et al. Expression of alpha-methylacyl-CoA racemase in papillary renal cell carcinoma. *Am J Surg Pathol*. 2004;28(1):69–76.

132. Lin F, Zhang PL, Yang XJ, et al. Human kidney injury molecule-1 (hKIM-1): a useful immunohistochemical marker for diagnosing renal cell carcinoma and ovarian clear cell carcinoma. *Am J Surg Pathol*. 2007;31(3):371–381.

133. Lau SK, Weiss LM, Chu PG. D2-40 immunohistochemistry in the differential diagnosis of seminoma and embryonal carcinoma: a comparative immunohistochemical study with KIT (CD117) and CD30. *Mod Pathol*. 2007;20(3):320–325.

134. Lau SK, Weiss LM, Chu PG. Differential expression of MUC1, MUC2, and MUC5AC in carcinomas of various sites: an immunohistochemical study. *Am J Clin Pathol*. 2004;122(1):61–69.

135. Tateyama H, Eimoto T, Tada T, Hattori H, Murase T, Takino H. Immunoreactivity of a new CD5 antibody with normal epithelium and malignant tumors including thymic carcinoma. *Am J Clin Pathol*. 1999;111(2):235–240.

136. Cheuk W, Kwan MY, Suster S, Chan JK. Immunostaining for thyroid transcription factor 1 and cytokeratin 20 aids the distinction of small cell carcinoma from Merkel cell carcinoma, but not pulmonary from extrapulmonary small cell carcinomas. *Arch Pathol Lab Med*. 2001;125(2):228–231.

137. Chu PG, Arber DA, Weiss LM. Expression of T/NK-cell and plasma cell antigens in nonhematopoietic epithelioid neoplasms: an immunohistochemical study of 447 cases. *Am J Clin Pathol*. 2003;120(1):64–70.

138. Kandil DH, Cooper K. Glypican-3: a novel diagnostic marker for hepatocellular carcinoma and more. *Adv Anat Pathol*. 2009;16(2):125–129.

139. Folpe AL, Hill CE, Parham DM, O'Shea PA, Weiss SW. Immunohistochemical detection of FLI-1 protein expression: a study of 132 round cell tumors with emphasis on CD99-positive mimics of Ewing's sarcoma/primitive neuroectodermal tumor. *Am J Surg Pathol*. 2000;24(12):1657–1662.

140. Chen ZM, Wang HL. Alteration of cytokeratin 7 and cytokeratin 20 expression profile is uniquely associated with tumorigenesis of primary adenocarcinoma of the small intestine. *Am J Surg Pathol*. 2004;28(10):1352–1359.

141. Zhang MQ, Lin F, Hui P, Chen ZM, Ritter JH, Wang HL. Expression of mucins, SIMA, villin, and CDX2 in small-intestinal adenocarcinoma. *Am J Clin Pathol*. 2007;128(5):808–816.

142. Mac MT, Chung F, Lin F, Hui P, Balzer BL, Wang HL. Expression of hepatocyte antigen in small intestinal epithelium and adenocarcinoma. *Am J Clin Pathol*. 2009;132(1):80–85.

143. Brown AF, Sirohi D, Fukuoka J, et al. Tissue-preserving antibody cocktails to differentiate primary squamous cell carcinoma, adenocarcinoma, and small cell carcinoma of lung. *Arch Pathol Lab Med*. 2013;137(9):1274–1281.

144. Travis WD, Brambilla E, Noguchi M, et al. Diagnosis of lung cancer in small biopsies and cytology: implications of the 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification. *Arch Pathol Lab Med*. 2013;137(5):668–684.

145. Nonaka D. A study of ΔNp63 expression in lung non-small cell carcinomas. *Am J Surg Pathol*. 2012;36(6):895–899.

146. Bishop JA, Teruya-Feldstein J, Westra WH, Pelosi G, Travis WD, Rekhtman N. p40 (ΔNp63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. *Mod Pathol*. 2012;25(3):405–415.

147. Butnor KJ, Burchette JL. p40 (ΔNp63) and keratin 34βE12 provide greater diagnostic accuracy than p63 in the evaluation of small cell lung carcinoma in small biopsy samples. *Hum Pathol*. 2013;44(8):1479–1486.

148. Zhang PJ, Goldblum JR, Pawel BR, Fisher C, Pasha TL, Barr FG. Immunophenotype of desmoplastic small round cell tumors as detected in cases with EWS-WT1 gene fusion product. *Mod Pathol*. 2003;16(3):229–235.

149. Tsuta K, Kato Y, Tochigi N, et al. Comparison of different clones (WT49 versus 6F-H2) of WT-1 antibodies for immunohistochemical diagnosis of malignant pleural mesothelioma. *Appl Immunohistochem Mol Morphol*. 2009;17(2):126–130.

150. Hwang H, Quenneville L, Yaziji H, Gown AM. Wilms tumor gene product: sensitive and contextually specific marker of serous carcinomas of ovarian surface epithelial origin. *Appl Immunohistochem Mol Morphol*. 2004;12(2):122–126.

151. Euscher ED, Malpica A, Deavers MT, Silva EG. Differential expression of WT-1 in serous carcinomas in the peritoneum with or without associated serous carcinoma in endometrial polyps. *Am J Surg Pathol*. 2005;29(8):1074–1078.

152. Koo J, Mertens RB, Mirocha JM, Wang HL, Dhall D. Value of islet 1 and PAX8 in identifying metastatic neuroendocrine tumors of pancreatic origin. *Mod Pathol*. 2012;25(6):893–901.

153. Graham RP, Shrestha B, Caron BL, et al. Islet-1 is a sensitive but not entirely specific marker for pancreatic neuroendocrine neoplasms and their metastases. *Am J Surg Pathol*. 2013;37(3):399–405.

154. Miettinen M, Wang ZF, Paetau A, et al. ERG transcription factor as an immunohistochemical marker for vascular endothelial tumors and prostatic carcinoma. *Am J Surg Pathol*. 2011;35(3):432–441.

155. Yaskiv O, Rubin BP, He H, Falzarano S, Magi-Galluzzi C, Zhou M. ERG protein expression in human tumors detected with a rabbit monoclonal antibody. *Am J Clin Pathol*. 2012;138(6):803–810.

156. Liu H, Shi J, Wilkerson M, Yang XJ, Lin F. Immunohistochemical evaluation of ERG expression in various benign and malignant tissues. *Ann Clin Lab Sci*. 2013;43(1):3–9.

157. Miettinen M, Wang Z, Sarlomo-Rikala M, Abdullaev Z, Pack SD, Fetsch JF. ERG expression in epithelioid sarcoma: a diagnostic pitfall. *Am J Surg Pathol*. 2013;37(10)1580–1585.

158. DeLair D, Oliva E, Köbel M, Macias A, Gilks CB, Soslow RA. Morphologic spectrum of immunohistochemically characterized clear cell carcinoma of the ovary: a study of 155 cases. *Am J Surg Pathol*. 2011;35(1):36–44.

159. Brimo F, Herawi M, Sharma R, Netto GJ, Epstein JI, Illei PB. Hepatocyte nuclear factor-1β expression in clear cell adenocarcinomas of the bladder and urethra: diagnostic utility and implications for histogenesis. *Hum Pathol*. 2011;42(11):1613–1619.

160. Fadare O, Liang SX. Diagnostic utility of hepatocyte nuclear factor 1-beta immunoreactivity in endometrial carcinomas: lack of specificity for endometrial clear cell carcinoma. *Appl Immunohistochem Mol Morphol*. 2012;20(6):580–587.

161. Doyle LA, Möller E, Dal Cin P, Fletcher CD, Mertens F, Hornick JL. MUC4 is a highly sensitive and specific marker for low-grade fibromyxoid sarcoma. *Am J Surg Pathol*. 2011;35(5):733–741.

162. Doyle LA, Wang WL, Dal Cin P, et al. MUC4 is a sensitive and extremely useful marker for sclerosing epithelioid fibrosarcoma: association with FUS gene rearrangement. *Am J Surg Pathol*. 2012;36(10):1444–1451.

163. Terry J, Saito T, Subramanian S, et al. TLE1 as a diagnostic immunohistochemical marker for synovial sarcoma emerging from gene expression profiling studies. *Am J Surg Pathol*. 2007;31(2):240–246.

164. Kosemehmetoglu K, Vrana JA, Folpe AL. TLE1 expression is not specific for synovial sarcoma: a whole section study of 163 soft tissue and bone neoplasms. *Mod Pathol*. 2009;22(7):872–878.

165. Jagdis A, Rubin BP, Tubbs RR, Pacheco M, Nielsen TO. Prospective evaluation of TLE1 as a diagnostic immunohistochemical marker in synovial sarcoma. *Am J Surg Pathol*. 2009;33(12):1743–1751.

166. Zhong M, De Angelo P, Osborne L, et al. Dual-color, break-apart FISH assay on paraffin-embedded tissues as an adjunct to diagnosis of Xp11 translocation renal cell carcinoma and alveolar soft part sarcoma. *Am J Surg Pathol*. 2010;34(6):757–766.

167. Williams A, Bartle G, Sumathi VP, et al. Detection of ASPL/TFE$_3$ fusion transcripts and the TFE3 antigen in formalin-fixed, paraffin-embedded tissue in a series of 18 cases of alveolar soft part sarcoma: useful diagnostic tools in cases with unusual histological features. *Virchows Arch*. 2011;458(3):291–300.

168. Busam KJ, Kucukgöl D, Sato E, Frosina D, Teruya-Feldstein J, Jungbluth AA. Immunohistochemical analysis of novel monoclonal antibody PNL2 and comparison with other melanocyte differentiation markers. *Am J Surg Pathol*. 2005;29(3):400–406.

169. Sangoi AR, McKenney JK, Brooks JD, Higgins JP. Evaluation of SF-1 expression in testicular germ cell tumors: a tissue microarray study of 127 cases. *Appl Immunohistochem Mol Morphol*. 2013;21(4):318–321.

170. Enriquez ML, Lal P, Ziober A, Wang L, Tomaszewski JE, Bing Z. The use of immunohistochemical expression of SF-1 and EMA in distinguishing adrenocortical tumors from renal neoplasms. *Appl Immunohistochem Mol Morphol*. 2012;20(2):141–145.

171. Akpalo H, Lange C, Zustin J. Discovered on gastrointestinal stromal tumour 1 (DOG1): a useful immunohistochemical marker for diagnosing chondroblastoma. *Histopathology*. 2012;60(7):1099–1106.

172. Miettinen M, Wang ZF, Lasota J. DOG1 antibody in the differential diagnosis of gastrointestinal stromal tumors: a study of 1840 cases. *Am J Surg Pathol*. 2009;33(9):1401–1408.

173. Ardeleanu C, Arsene D, Hinescu M, et al. Pancreatic expression of DOG1: a novel gastrointestinal stromal tumor (GIST) biomarker. *Appl Immunohistochem Mol Morphol*. 2009;17(5):413–418.

174. Espinosa I, Lee CH, Kim MK, et al. A novel monoclonal antibody against DOG1 is a sensitive and specific marker for gastrointestinal stromal tumors. *Am J Surg Pathol*. 2008;32(2):210–218.

175. Hirokawa M, Carney JA. Cell membrane and cytoplasmic staining for MIB-1 in hyalinizing trabecular adenoma of the thyroid gland. *Am J Surg Pathol*. 2000;24(4):575–578.
176. Casey MB, Sebo TJ, Carney JA. Hyalinizing trabecular adenoma of the thyroid gland identification through MIB-1 staining of fine-needle aspiration biopsy smears. *Am J Clin Pathol*. 2004;122(4):506–510.
177. Pollack SM, Jungbluth AA, Hoch BL, et al. NY-ESO-1 is a ubiquitous immunotherapeutic target antigen for patients with myxoid/round cell liposarcoma. *Cancer*. 2012;118(18):4564–4570.
178. Hemminger JA, Iwenofu OH. NY-ESO-1 is a sensitive and specific immunohistochemical marker for myxoid and round cell liposarcomas among related mesenchymal myxoid neoplasms. *Mod Pathol*. 2013;26(9):1204–1210.
179. L Hoang L, Qi W, Bremer R, et al. A newly developed anti-uroplakin II antibody with increased sensitivity in urothelial carcinoma of the bladder [USCAP abstract 901]. *Mod Pathol*. 2013;26(S2):218A.
180. Luong-Player A, Liu H, Wang HL, Lin F. CA IX expression in various tumors. *Am J Clin Pathol*. 2014;141(2):219–225.
181. Fujiwara S, Nawa A, Nakanishi T, et al. Thyroid transcription factor 1 expression in ovarian carcinomas is an independent prognostic factor. *Hum Pathol*. 2010;41(4):560–565.
182. Zhang PJ, Gao HG, Pasha TL, Litzky L, Livolsi VA. TTF-1 expression in ovarian and uterine epithelial neoplasia and its potential significance, an immunohistochemical assessment with multiple monoclonal antibodies and different secondary detection systems. *Int J Gynecol Pathol*. 2009;28(1):10–18.
183. Gu K, Shah V, Ma C, Zhang L, Yang M. Cytoplasmic immunoreactivity of thyroid transcription factor-1 (clone 8G7G3/1) in hepatocytes: true positivity or cross-reaction? *Am J Clin Pathol*. 2007;128(3):382–388.
184. Pereira TC, Share SM, Magalhães AV, Silverman JF. Can we tell the site of origin of metastatic squamous cell carcinoma?: an immunohistochemical tissue microarray study of 194 cases. *Appl Immunohistochem Mol Morphol*. 2011; 19(1):10–14.
185. Burch JB. Regulation of GATA gene expression during vertebrate development. *Semin Cell Dev Biol*. 2005;16(1):71–81.
186. Zheng R, Blobel GA. GATA transcription factors and cancer. *Genes Cancer*. 2010;1(12):1178–1188.
187. Yoon NK, Maresh EL, Shen D, et al. Higher levels of GATA3 predict better survival in women with breast cancer. *Hum Pathol*. 2010;41(12):1794–1801.
188. Hoch RV, Thompson DA, Baker RJ, Weigel RJ. GATA-3 is expressed in association with estrogen receptor in breast cancer. *Int J Cancer*. 1999;84(2):122–128.
189. Ciocca V, Daskalakis C, Ciocca RM, Ruiz-Orrico A, Palazzo JP. The significance of GATA3 expression in breast cancer: a 10-year follow-up study. *Hum Pathol*. 2009;40(4):489–495.
190. Jacquemier J, Charafe-Jauffret E, Monville F, et al. Association of GATA3, P53, Ki67 status and vascular peritumoral invasion are strongly prognostic in luminal breast cancer. *Breast Cancer Res*. 2009;11(2):R23. doi:10.1186/bcr2249.
191. Liu H, Shi J, Prichard JW, Gong Y, Lin F. Immunohistochemical evaluation of GATA3 expression in ER-negative breast carcinomas. *Am J Clin Pathol*. 2014; 141(5):648–665.
192. Onuma K, Dabbs DJ, Bhargava R. Mammaglobin expression in the female genital tract: immunohistochemical analysis in benign and neoplastic endocervix and endometrium. *Int J Gynecol Pathol*. 2008;27(3):418–425.
193. Ren JL, Luo JY, Lu YP, Wang L, Shi HX. Molecular forms of trefoil factor 1 in normal gastric mucosa and its expression in normal and abnormal gastric tissues. *World J Gastroenterol*. 2006;12(45):7361–7364.
194. Mori K, Fujii R, Kida N, et al. Complete primary structure of the human estrogen-responsive gene (pS2) product. *J Biochem*. 1990;107(1):73–76.
195. Im S, Yoo C, Jung JH, Choi HJ, Yoo J, Kang CS. Reduced expression of TFF1 and increased expression of TFF3 in gastric cancer: correlation with clinicopathological parameters and prognosis. *Int J Med Sci*. 2013;10(2):133–140.
196. Lin F, Shi J, Zhu S, et al. Cadherin-17 and SATB2 are sensitive and specific immunomarkers for medullary carcinoma of the large intestine [published online ahead of print January 17, 2014]. *Arch Pathol Lab Med*. doi:10.5858/arpa.2013-0452-OA.
197. Hinoi T, Tani M, Lucas PC, et al. Loss of CDX2 expression and microsatellite instability are prominent features of large cell minimally differentiated carcinomas of the colon. *Am J Pathol*. 2001;159(6):2239–2248.
198. Winn B, Tavares R, Fanion J, et al. Differentiating the undifferentiated: immunohistochemical profile of medullary carcinoma of the colon with an emphasis on intestinal differentiation. *Hum Pathol*. 2009;40(3):398–404.
199. McGregor DK, Wu TT, Rashid A, Luthra R, Hamilton SR. Reduced expression of cytokeratin 20 in colorectal carcinomas with high levels of microsatellite instability. *Am J Surg Pathol*. 2004;28(6):712–718.
200. Li Z, Zhou K, Mei K, Kang Q, Cao D. SATB2 is a highly sensitive marker for hindgut well-differentiated neuroendocrine tumors [USCAP abstract 682]. *Mod Patho*l. 2013;26(S2):164A.
201. Kakar S, Gown AM, Goodman ZD, Ferrell LD. Best practices in diagnostic immunohistochemistry: hepatocellular carcinoma versus metastatic neoplasms. *Arch Pathol Lab Med*. 2007;131(11):1648–1654.
202. Yim SH, Chung YJ. An overview of biomarkers and molecular signatures in HCC. *Cancers*. 2010;2(2)809–823.
203. Shafizadeh N, Ferrell LD, Kakar S. Utility and limitations of glypican-3 expression for the diagnosis of hepatocellular carcinoma at both ends of the differentiation spectrum. *Mod Pathol*. 2008;21(8):1011–1018.
204. Lorenzo PI, Jimenez Moreno CM, Delgado I, et al. Immunohistochemical assessment of Pax8 expression during pancreatic islet development and in human neuroendocrine tumors. *Histochem Cell Biol*. 2011;136(5):595–607.
205. Kaelin WG Jr. Molecular basis of the VHL hereditary cancer syndrome. *Nat Rev Cancer*. 2002;2(9):673–682.
206. Kim WY, Kaelin WG Jr. Molecular pathways in renal cell carcinoma—rationale for targeted treatment. *Semin Oncol*. 2006;33(5):588–595.
207. Sudarshan S, Linehan WM. Genetic basis of cancer of the kidney. *Semin Oncol*. 2006;33(5):544–551.
208. Deisch J, Raisanen J, Rakheja D. Immunoexpression of SALL4 in Wilms tumors and developing kidney. *Pathol Oncol Res*. 2011;17(3):639–644.
209. Deisch J, Raisanen J, Rakheja D. Immunohistochemical expression of embryonic stem cell markers in malignant rhabdoid tumors. *Pediatr Dev Pathol*. 2011;14(5):353–359.
210. Alexander RE, Grignon DJ, Cheng L, Idrees MT. Cytoplasmic OCT4 staining is a sensitive and specific marker of neuroendocrine differentiation [USCAP abstract 532]. *Mod Pathol*; 2013;26(S2):129A.
211. Alexander RE, Cheng L, Grignon DJ, Idrees M. Cytoplasmic staining of OCT4 is a highly sensitive marker of adrenal medullary-derived tissue. *Am J Surg Pathol*. 2013;37(5):727–733.
212. Liu A, Cheng L, Du J, et al. Diagnostic utility of novel stem cell markers SALL4, OCT4, NANOG, SOX2, UTF1, and TCL1 in primary mediastinal germ cell tumors. *Am J Surg Pathol*. 2010;34(5):697–706.
213. Al-Agha OM, Huwait HF, Chow C, et al. FOXL2 is a sensitive and specific marker for sex cord-stromal tumors of the ovary. *Am J Surg Pathol*. 2011;35(4): 484–494.
214. Desouki NN, Kallas SJ, Hameed O, Khabele D, Crispens MA, Fadare O. Differential vimentin expression in ovarian and endometrial endometrioid adenocarcinomas: diagnostic utility in distinguishing double primaries from metastatic tumors [USCAP abstract 1127]. *Mod Pathol*; 2013;26(S2):271A.
215. Busam KJ, Jungbluth AA, Rekthman N, et al. Merkel cell polyomavirus expression in Merkel cell carcinomas and its absence in combined tumors and pulmonary neuroendocrine carcinomas. *Am J Surg Pathol*. 2009;33(9):1378–1385.
216. Wick MR, Vitsky JL, Ritter JH, Swanson PE, Mills SE. Sporadic medullary carcinoma of the colon: a clinicopathologic comparison with nonhereditary poorly differentiated enteric-type adenocarcinoma and neuroendocrine colorectal carcinoma. *Am J Clin Pathol*. 2005;123(1):56–65.
217. Chan ES, Alexander J, Swanson PE, Jain D, Yeh MM. PDX-1, CDX-2, TTF-1, and CK7: a reliable immunohistochemical panel for pancreatic neuroendocrine neoplasms. *Am J Surg Pathol*. 2012;36(5):737–743.
218. Hermann G, Konukiewitz B, Schmitt A, Perren A, Klöppel G. Hormonally defined pancreatic and duodenal neuroendocrine tumors differ in their transcription factor signatures: expression of ISL1, PDX1, NGN3, and CDX2. *Virchows Arch*. 2011;459(2):147–154.
219. Abdul-Al HM, Wang G, Makhlouf HR, Goodman ZD. Fibrolamellar hepatocellular carcinoma: an immunohistochemical comparison with conventional hepatocellular carcinoma. *Int J Surg Pathol*. 2010;18(5):313–318.
220. Gurel B, Ali TZ, Montgomery EA, et al. NKX3.1 as a marker of prostatic origin in metastatic tumors. *Am J Surg Pathol*. 2010;34(8):1097–1105.
221. Gelmann EP, Bowen C, Bubendorf L. Expression of NKX3.1 in normal and malignant tissues. *Prostate*. 2003;55(2):111–117.
222. Yoshida A, Sekine S, Tsuta K, Fukayama M, Furuta K, Tsuda H. NKX2.2 is a useful immunohistochemical marker for Ewing sarcoma. *Am J Surg Pathol*. 2012;36(7):993–999.
223. Igbokwe A, Lopez-Terrada DH. Molecular testing of solid tumors. *Arch Pathol Lab Med*. 2011;135(1):67–82.
224. Ma XJ, Patel R, Wang X, et al. Molecular classification of human cancers using a 92-gene real-time quantitative polymerase chain reaction assay. *Arch Pathol Lab Med*. 2006;130(4):465–473.
225. Hainsworth JD, Rubin MS, Spigel DR, et al. Molecular gene expression profiling to predict the tissue of origin and direct site-specific therapy in patients with carcinoma of unknown primary site: a prospective trial of the Sarah Cannon Research Institute. *J Clin Oncol*. 2013;31(2):217–223.
226. Rosenfeld N, Aharonov R, Meiri E, et al. MicroRNAs accurately identify cancer tissue origin. *Nat Biotechnol*. 2008;26(4):462–469.
227. Handorf CR, Kulkarni A, Grenert JP, et al. A multicenter study directly comparing the diagnostic accuracy of gene expression profiling and immunohistochemistry for primary site identification in metastatic tumors. *Am J Surg Pathol*. 2013;37(7):1067–1075.